INTERNATIONAL JOURNAL of SYSTEMATIC BACTERIOLOGY
October 1972, p. 193-200
Copyright © 1972 International Association of Microbiological Societies
Vol. 22, No. 4
Printed in U.S.A.

Differentiation of *Enterobacter aerogenes* from Klebsiellae by Deoxyribonucleic Acid Reassociation

DON J. BRENNER, A. G. STEIGERWALT, and G. R. FANNING

Division of Biochemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C. 20012

Polynucleotide sequence relatedness tests were carried out to determine the extent of deoxyribonucleic acid (DNA) divergence among species of *Klebsiella* and *Enterobacter aerogenes* strains. Labeled, denatured DNA fragments from *K. pneumoniae* type 2 and *E. aerogenes* 1627-66 were each incubated with an excess of unlabeled DNA fragments from *Klebsiella* species and strains of *E. aerogenes*. Reassociated DNA duplexes were separated from unreacted DNA on hydroxyapatite. The stability of reassociated DNA duplexes was determined in a series of thermal elutions from hydroxyapatite. Relative reassociation of DNA from 5 *Klebsiella* strains to *K. pneumoniae* type 2 was 80 to 91%. In no case did the related DNA duplexes exhibit evidence of greater than 1.2% unpaired bases. Similarly, DNA from 10 strains of *E. aerogenes* exhibited 83 to 100% relative reassociation with DNA from *E. aerogenes* 1627-66. In this case, 2.2% was the largest amount of unpaired bases within a reassociated DNA duplex. Conversely, only 56% relative relatedness was observed in 18 reactions between klebsiellae and *E. aerogenes*. In these reactions, related DNA duplexes exhibited an average of 9% unpaired bases. We conclude that klebsiellae and *E. aerogenes* each are highly related groups of strains and that these two groups have diverged significantly from one another. An *E. cloacae* strain exhibited some 40% relative relatedness with some 12% unpaired bases in reactions with *K. pneumoniae* and *E. aerogenes*.

Before the widespread use of antibiotics, *Klebsiella* and *Enterobacter* species were rarely recognized as primary pathogens (15). These organisms are now increasingly important causes of nosocomial infection, especially urinary tract infections and bacteremias (15). Despite the increased occurrence of these organisms, many clinical laboratories have not attempted, and do not now attempt, to differentiate between klebsiellae and enterobacters (3, 18). Part of the difficulty lies in the taxonomic and nomenclatural confusion surrounding *K. pneumoniae* and *E. aerogenes* and in a former lack of reliable methods for distinguishing between these organisms. According to Graber (18), many laboratories make no attempt to distinguish between *Klebsiella* and *Enterobacter*, whereas other laboratories allow the source of isolation to determine the designation. Strains isolated from sputum are arbitrarily placed in *Klebsiella*, and strains isolated from feces or urine are assigned to *Enterobacter*. Still other laboratories rely on the colony type: mucoid colonies are designated klebsiellae and nonmucoid colonies are reported as enterobacters.

Of course much of the confusion in nomenclature of these organisms is due to this practice of using one name for an organism isolated from urine and another name for the identical organism isolated from sputum (15). Any rational taxonomy and nomenclature must be based on the premise that strains of a specific organism are genotypically identical regardless of their source of isolation.

The current practices used in identifying these organisms hinder epidemiological studies. In addition, the course of antibiotic therapy is affected as antibiotic susceptibility patterns differ among species of *Klebsiella* and *Enterobacter*. Finally, if differentiation is not made, the clinician may dismiss a report of *Enterobacter* as a contaminant of the sputum (18).

The two most difficult species of the tribe *Klebsiellae* to differentiate are *K. pneumoniae* and *E. aerogenes*. It appears that typical strains

of these species are distinguishable only on the basis of motility, ornithine decarboxylase, urease, and perhaps resistance to cephalosporins. The present study was designed to assess deoxyribonucleic acid (DNA) relatedness among *Klebsiella* species and to determine whether the difficulties in distinguishing between klebsiellae and *E. aerogenes* on the basis of phenotype are reflected in a high level of DNA relatedness. The answers to these questions should provide an approach to resolving the problems encountered in the systematics of this group.

MATERIALS AND METHODS

Organisms, media, and DNA preparation. The bacteria used in this study are listed in Table 1. The organisms were maintained on brain heart infusion agar and were cultivated on brain heart infusion. A modified C medium (10) was used in the preparation of labeled DNA. The preparation of labeled and nonlabeled DNA has been described (10, 12).

DNA reassociation. The conditions employed for DNA reassociation and separation of single-stranded from reassociated DNA on hydroxyapatite (HA) are similar to those described previously (10). These techniques, with current modifications, are described below.

A 0.1-μg amount of ^{32}P-labeled DNA per ml is added to 150 μg/ml of each desired nonlabeled DNA contained in 0.28 M PB (phosphate buffer, an equimolar mixture of NaH_2PO_4 and Na_2HPO_4, *p*H 6.8). The DNA mixtures are denatured by heating in a boiling-water bath for 3 to 4 min and then immediately cooled in an ice bath. The samples are incubated for 16 hr at 60 or 75 C. These conditions permit essentially complete reassociation of unlabeled DNA fragments with each other or with labeled DNA fragments but preclude significant reassociation of labeled DNA fragments with one another. Reaction mixtures are either diluted with distilled water to 0.14 M PB concentration and immediately added to HA (DNA grade, Bio-Gel, HTP, Bio-Rad Laboratories, Richmond, Calif.) or frozen until use.

The three modifications in the incubation procedure are: (i) omission of 0.05 M ethylenediaminetetraacetic acid (EDTA) in the reaction mixture, (ii) increasing the buffer concentration from 0.14 M PB to 0.28 M PB, and (iii) decreasing the amount of unlabeled DNA from 400 μg/ml to 150 μg/ml. EDTA was initially added to incubation mixtures to preclude cation-dependent deoxyribonuclease activity, especially during the course of long-term incubations (48 hr or longer). Since we rarely, if ever, encounter DNA degradation dur-

TABLE 1. *Bacterial strains employed*

Organism	Strain	Source[a]
Enterobacter aerogenes	Standard strain, motile	Univ. of Washington
E. aerogenes	865-65 Motile	CDC
E. aerogenes	1494-70 Nonmotile	CDC
E. aerogenes	1627-66 Nonmotile	CDC
E. aerogenes	2164-70 Motile	CDC
E. aerogenes	2979-69 Motile	CDC
E. aerogenes	3006-67 Nonmotile	CDC
E. aerogenes	3254-70 Motile	CDC
E. aerogenes	3432-70 Motile	CDC
E. aerogenes	13048 Motile	ATCC
E. aerogenes	15038 Motile	ATCC
E. cloacae	Standard strain	WRAIR
Klebsiella edwardsii subsp. *edwardsii*	13886	ATCC
K. edwardsii subsp. *atlantae*	13887	ATCC
K. ozaenae		CDC
K. ozaenae	276-71	CDC
K. ozaenae	1660-71	CDC
K. pneumoniae	Type 1	CDC
K. pneumoniae	Type 2	CDC
K. pneumoniae	1385-71	CDC
K. pneumoniae	1460-71	CDC
K. pneumoniae	1515-71	CDC
K. pneumoniae	1627-71	CDC
K. pneumoniae	1736-71	CDC
K. pneumoniae	1799-71	CDC
K. rhinoscleromatis		CDC
K. rhinoscleromatis	895-68	CDC
K. rhinoscleromatis	4902-68	CDC

[a] CDC, Center for Disease Control, Atlanta, Ga.; ATCC, American Type Culture Collection, Rockville Md WRAIR, Walter Reed Army Institute of Research, Washington, D.C.

TABLE 2. *Reactions using labeled DNA from Klebsiella pneumoniae type 2*

Source of unlabeled DNA	Relative binding at 60 C (%)	$\Delta T_{m(e)}$[a] at 60 C	Relative binding at 75 C %	$\Delta T_{m(e)}$ at 75 C	TBI[b]
Klebsiella pneumoniae type 2	100[c]		100		
K. edwardsii subsp. *atlantae*	80	1.0	75	0.5	.94
K. edwardsii subsp. *edwardsii*	81	0.9	72	1.0	.89
K. ozaenae	80	1.2	74	0.5	.93
K. pneumoniae type 1	91	0.7	76	0.6	.80
K. rhinoscleromatis	85	0.4	77	0.4	.91
Enterobacter aerogenes	56	8.9	22	6.5	.39
E. aerogenes 865-65	51		16		.31
E. cloacae	37	11.8	10	6.5	.27

[a] $T_{m(e)}$, Thermal elution midpoint; that temperature at which 50% of the DNA bound to hydroxyapatite (at the 60 or 75 C incubation temperature) is eluted. $\Delta T_{m(e)}$ is the decrease in $T_{m(e)}$ between heterologous reactions and the homologous *K. pneumoniae* type 2 reaction. The $T_{m(e)}$ for *K. pneumoniae* type 2 in our system is 90 to 92 C.

[b] TBI, Thermal binding index; relative binding at 75 C divided by relative binding at 60 C.

[c] Binding before normalization was approximately 85%; heterologous DNA reassociation reactions were carried out three or more times.

ing the course of a 16- to 24-hr incubation, the use of EDTA was discontinued. DNA reassociates approximately 2.7 times as fast in 0.28 M PB as in 0.14 M PB (13). Therefore, by using 0.28 M PB, we economize on the amount of DNA. Control reactions indicate that completeness of reassociation in both homologous and heterologous reactions is not affected by these modifications.

Reassociated DNA is separated from unreacted DNA by adding the samples (diluted to 0.14 M PB) to HA by a batch method (11). HA is equilibrated with 0.14 M PB plus 0.4% sodium dodecyl sulfate (SDS) and held at the temperature at which the samples had been incubated (60 or 75 C). SDS is included to prevent nonspecific binding of DNA to HA. Reassociated DNA adsorbs to HA in this buffer. HA is washed four times with 15-ml portions of 0.14 M PB plus 0.4% SDS to remove unreassociated DNA fragments. DNA adsorbed to HA is washed with 15-ml portions of 0.14 M PB at temperatures increasing in increments of 2.5 C (reactions using ^{32}P-labeled DNA from *K. pneumoniae* type 2) or 5.0 C increments (reactions using ^{32}P-labeled DNA from *E. aerogenes*) to 100 C. As the temperature exceeds the dissociation temperature of DNA duplexes bound to HA, the resultant single-stranded DNA is eluted, and a thermal elution profile is obtained. HA is finally washed with 0.4 M PB to elute any material that remains bound to HA. Neither double- nor single-stranded DNA remains bound to HA in 0.4 M PB. When thermal stability is not of interest, the amount of heteroduplex formation is determined by eluting bound DNA in four 15-ml washes with 0.4 M PB. All eluates are collected and assayed for radioactivity.

RESULTS

DNA relatedness among a series of *Klebsiella* strains, two *E. aerogenes* strains, and an *E. cloacae* strain was assessed using a strain of *K. pneumoniae* type 2 as the arbitrarily chosen reference. Reactions were carried out at 60 and 75 C to determine relatedness at an optimal criterion for reassociation as well as at a stringent criterion at which only highly related DNA duplexes are stable. Thermal elution profiles were generated to compare the stability of heteroduplex DNA with that of homologous reassociated DNA from our strain of *K. pneumoniae* type 2. Homologous reassociated DNA species have a characteristic $T_{m(e)}$: that temperature at which 50% of the DNA duplexes bound to HA are eluted. The decrease in $T_{m(e)}$ in heterologous DNA duplexes can be correlated with the amount of unpaired bases within the heteroduplex. It has been calculated that each degree decreases in $T_{m(e)}$ is the result of from 0.5 to 1.5% unpaired bases (1, 6, 20). We have arbitrarily used an approximation of 1 degree C decrease in $T_{m(e)}$ per percent of unpaired bases.

Our strain of *K. pneumoniae* type 2 shares 80 to 90% of its genome with strains of *K. pneumoniae*, *K. ozaenae*, *K. rhinoscleromatis*, and *K. edwardsii* (Table 2). The $\Delta T_{m(e)}$ values for these reactions are between 0.4 C and 1.2 C, indicating a minimum amount of divergence in the related sequences. At the stringent 75 C incubation temperature, relative binding of *Klebsiella* strains remains high (72 to 77%). The thermal binding index (TBI), which is the ratio of relative binding at 75 C divided by relative binding at 60 C, is between 0.80 and 0.94. These values, approaching unity, indicate that the preponderance of sequences held in common under optimal (60 C) conditions contain few regions

that are not stable at the higher incubation criterion. $\Delta T_{m(e)}$ values from 75 C reactions remain very low.

Our strain of *K. pneumoniae* type 2 exhibited only 51 and 56% relatedness to two *E. aerogenes* strains. Some 9% divergence is present within these related sequences. In 75 C reactions, relatedness drops to about 20% with an accompanying increase in stability (less divergence) in those duplexes that remain stable. Less than 40% reaction occurs between DNA from *E. cloacae* and *K. pneumoniae* type 2. The stability of this reaction indicates 12% unpaired bases within the related sequences. At 75 C, only 10% relatedness is seen between these organisms.

Thermal elution profiles of representative *K. pneumoniae* type 2 reassociated DNA duplexes are shown in Fig. 1. In Fig. 1A, the homologous *K. pneumoniae* type 2 profile is seen to approximate a Gaussian distribution with maximum elution at 92.5 C and little evidence of sequences eluting below 80 C. The heterologous *Klebsiella* profiles also show elution maxima at 92.5 C. In these reactions, the shape of the curves is somewhat skewed toward lower stability and, in some cases, there is more DNA eluting below 80 C. In Fig. 1B, the elution profiles obtained from *K. pneumoniae-E. aerogenes* and *K. pneumoniae-E. cloacae* reactions reflect the diminished stability of these heteroduplexes. Both profiles are broad and non-Gaussian. The elution maximum is 82.5 C with *E. aerogenes* and 75 C with *E. cloacae*.

The results obtained with *K. pneumoniae* type 2 reference DNA indicate significant divergence between *E. aerogenes* and *K. pneumoniae*. Only two strains of *E. aerogenes* were tested, however, and these strains are motile. A nonmotile strain

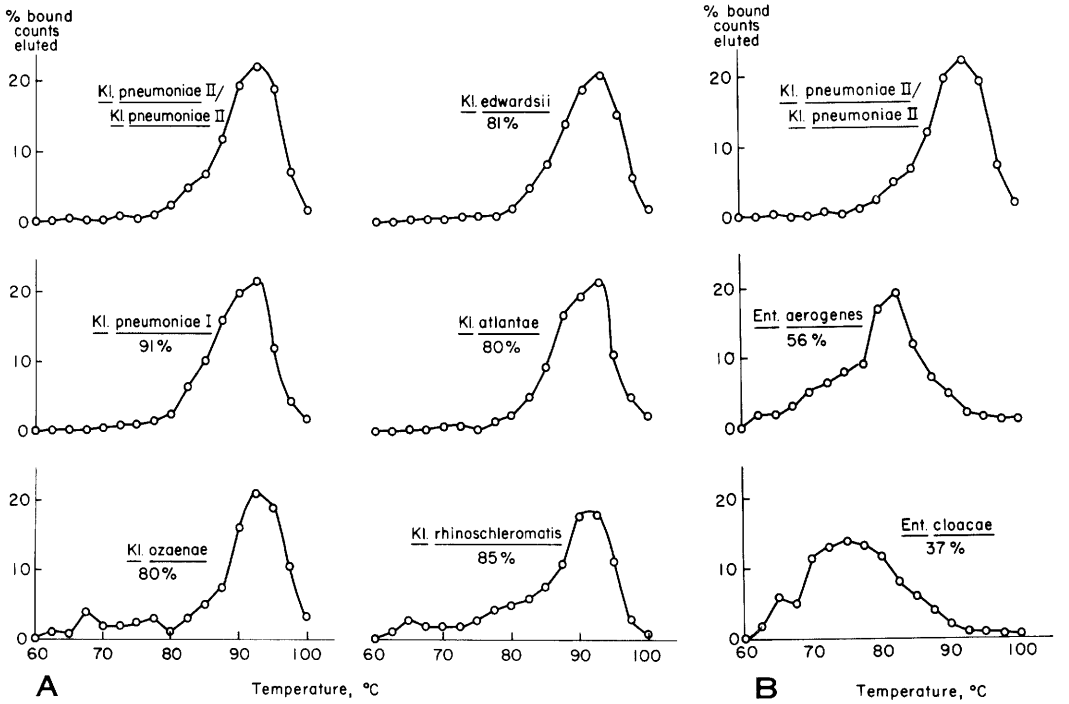

FIG. 1. *Thermal stability profiles of Klebsiella pneumoniae type 2 DNA duplexes.* ^{32}*P-labeled DNA from K. pneumoniae type 2 was incubated with a series of unlabeled DNA preparations. Incubations were carried out at 60 C in 0.28 M phosphate buffer (PB). The samples were diluted to 0.14 M PB and passed through hydroxyapatite (HA). The duplexes adsorbed to HA were eluted in a series of washes with 0.14M PB at increasing 2.5 C increments to 100 C and then with 0.4 M PB (values not shown). Binding in the homologous K pneumoniae type 2 reaction was arbitrarily designated 100%. The percentages given represent the relative binding of labeled K. pneumoniae DNA to DNA from the strains designated. A, Thermal stability profiles of DNA duplexes formed between K. pneumoniae type 2 and Klebsiella strains. B, Thermal stability profiles of DNA duplexes formed between K pneumoniae type 2 and Enterobacter strains.* $T_{m(e)}$ *values were calculated from integral curves. The* $T_{m(e)}$ *value corresponds exactly to the elution peak in the differential elution profiles only when the elution profile is completely Gaussian.*

TABLE 3. *Reactions using labeled DNA from nonmotile Enterobacter aerogenes strain 1627-66*

Source of unlabeled DNA	Relative[a] binding at 60 C (%)	$\Delta T_{m(e)}$ at 60 C	Relative binding at 75 C (%)	$\Delta T_{m(e)}$ at 75 C	TBI[a]
Enterobacter aerogenes 1627-66 nonmotile	100[b]		100		
E. aerogenes 1494-70 nonmotile	89	0.4	92	0.8	1.03
E. aerogenes 3006-67 nonmotile	100	0.2	99		0.99
E. aerogenes motile	88	0.6	88	0.4	1.00
E. aerogenes 865-65 motile	94	1.8	90	1.8	0.96
E. aerogenes 2164-70 motile	98	0.0	99	0.1	1.01
E. aerogenes 2979-69 motile	87	0.5	87	0.3	1.00
E. aerogenes 3254-70 motile	98	0.0	99		1.01
E. aerogenes 3432-70 motile	83	2.2	84	2.6	1.01
E. aerogenes 13048 motile	91	0.2	90	0.7	0.99
E. aerogenes 15038 motile	89	1.3	87	0.8	0.98
E. cloacae	44	12.3	19	7.2	0.43
Klebsiella edwardsii subsp. *atlantae*	57	10.0	38	5.5	0.67
K. edwardsii subsp. *edwardsii*	55	9.5	34	5.3	0.62
K. ozaenae	59	9.0	37	5.1	0.63
K. ozaenae 276-71	61	8.1	38	5.8	0.63
K. ozaenae 1660-71	53	9.2	31	6.7	0.58
K. pneumoniae type 1	55	9.6	42	5.4	0.76
K. pneumoniae type 2	64	8.8	45	5.0	0.70
K. pneumoniae 1385-71	56	9.6	40	6.2	0.71
K. pneumoniae 1460-71	59	9.1	48	6.8	0.81
K. pneumoniae 1515-71	47	8.4	38	6.7	0.81
K. pneumoniae 1627-71	58	8.6	45	6.3	0.78
K. pneumoniae 1736-71	53	9.0	39	5.2	0.74
K. pneumoniae 1799-71	58	9.2	39	7.0	0.67
K. rhinoscleromatis	51				
K. rhinoscleromatis 895-68	53	9.0	44		0.83
K. rhinoscleromatis 4902-68	58	7.4	38	6.0	0.66

[a] See Table 2 for definitions of $\Delta T_{m(e)}$ and TBI.

[b] Binding before normalization was approximately 85%, and the $T_{m(e)}$ of these reassociated sequences was 90 to 92 C.

of *E. aerogenes* (1627-66) was therefore chosen as the reference strain to detect any gross differences between motile and nonmotile strains of *E. aerogenes* and to assess relatedness of *Klebsiella* strains to a nonmotile *E. aerogenes* strain.

The *E. aerogenes* strains formed a closely related group in which virtually identical relatedness values are obtained from both 60 C and 75 C reactions (Table 3). Relatedness values are between 83 and 100%, with a mean relative relatedness of 92% to *E. aerogenes* 1627-66. $\Delta T_{m(e)}$ values are from 0 to 2.2 C and indicate less than 1% divergence within related sequences in 7 of the 10 strains tested. At 60 C, reactions of *E. aerogenes* 1627-66 DNA with DNA from 16 strains of *Klebsiella* species show a mean 56% relative relatedness with a range between 47% and 64%. $\Delta T_{m(e)}$ values were indicative of 7.5 to 10.0% unpaired bases within the related sequences. At 75 C, these klebsiellae showed 31 to 48% relative relatedness to *E. aerogenes* 1627-66. In some cases as much as 80% of the sequences formed at 60 C were stable in a 75 C incubation. *E. cloacae* exhibits about the same level of relatedness (44%) and unpaired bases (12%) to *E. aerogenes* as to *K. pneumoniae*. At 75 C, there is a significant decrease in relatedness and increase in thermal stability of those sequences still held in common between *E. cloacae* and *E. aerogenes*.

Thermal elution profiles representative of *E. aerogenes* reactions are shown in Fig. 2. These profiles are similar to those in Fig. 1, except that elutions were carried out at 5.0 C increments instead of 2.5 C increments. As expected, profiles from heterologous *E. aerogenes* reactions are quite similar in stability to the homologous *E. aerogenes* 1627-66 DNA profile. Elution maxima are at 95 C (there was no elution at 92.5 C), and there is little indication of DNA eluting below 80 C. In contrast, the *E. aerogenes-Klebsiella* reactions yield broader, less stable pro-

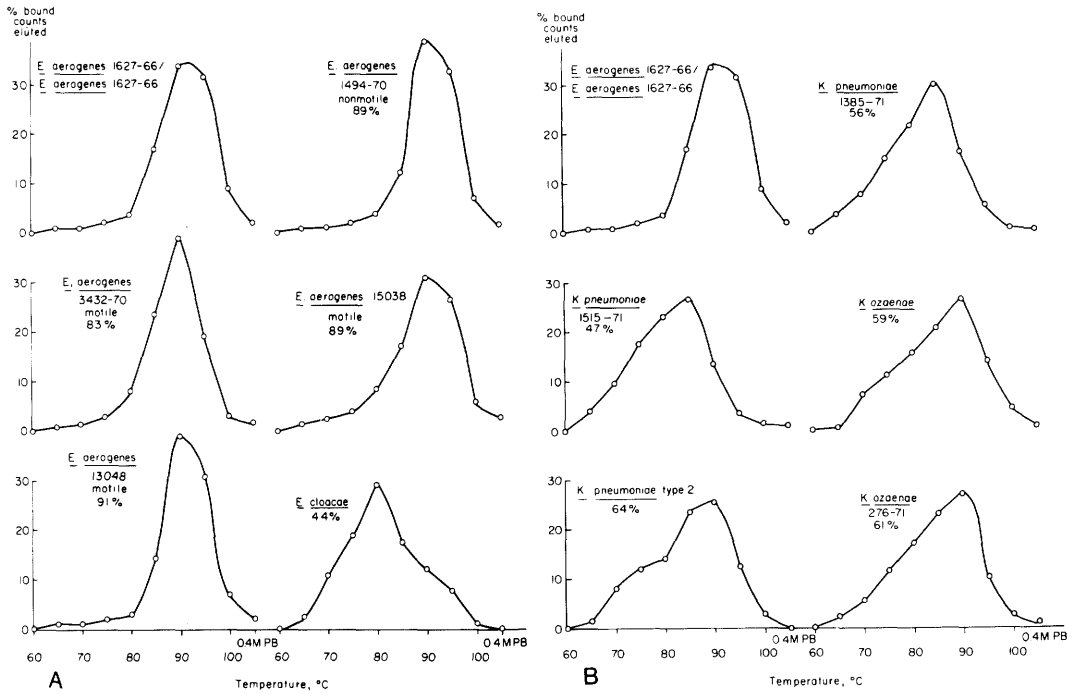

FIG. 2. *Thermal stability profiles of Enterobacter aerogenes strain 1627-66 DNA duplexes. The procedure is identical to that described for Fig. 1 except that DNA duplexes were eluted from hydroxyapatite (HA) with washes at increasing 5.0 C increments; the final 0.4 M PB elution values are shown. A, Thermal stability profiles of DNA duplexes formed between E. aerogenes strain 1627-66 and Enterobacter strains. B, Thermal stability profiles of DNA duplexes formed between E. aerogenes strain 1627-66 and Klebsiella strains.*

files with significant DNA eluting at lower temperatures. The least stable profile is that from the *E. aerogenes-E. cloacae* reaction.

DISCUSSION

In our experience, a series of closely related bacteria exhibits 75% or greater DNA relatedness. The thermal stability of DNA duplexes formed between these strains is usually less than 3 C below that of homologous DNA duplexes. This pattern has been observed in *Neisseria* species (19), *Escherichia coli* strains (12), and *Shigella* species (12; Brenner, *unpublished data*). Based on DNA relatedness and heteroduplex stability, the *Klebsiella* species studied are a highly related group of organisms. The same conclusion certainly is applicable to the *E. aerogenes* strains. Moreover, differences in motility do not affect gross DNA relatedness between *E. aerogenes* strains. Both extent of relatedness and stability of heteroduplexes indicate that klebsiellae and *E. aerogenes* strains belong to significantly different groups. The one *E. cloacae* strain tested has diverged significantly from both klebsiellae and *E. aerogenes*. Studies to be published separately imply that *E. cloacae*, *E. liquefaciens*, and *E. hafniae* each belong to different groups and that *E. liquefaciens* may more properly be placed in the genus *Serratia*.

The 50 to 65% relatedness observed between klebsiellae and *E. aerogenes* strains is somewhat higher than that observed between other members of the family *Enterobacteriaceae* (Table 4). In addition, the $\Delta T_{m(e)}$ values of 8 to 10 C are somewhat more stable than those usually observed between organisms exhibiting 40 to 55% reaction (9, 10; Table 4). Particularly striking is the 60 to 80% DNA related at 60 C that remains capable of forming stable duplexes at 75 C. It appears that, although one-third to one-half of the DNA has diverged to a point where it is no longer related at optimal reassociation conditions, the remaining DNA is conserved to a greater extent than normally observed between groups of enteric bacteria.

What bearing do these data have on taxonomy and clinical differentiation of klebsiellae and *E. aerogenes* strains? From the point of view of taxonomy, it is evident that one is not justified in distinguishing *E. aerogenes* strains based on

motility. Neither can one consider nonmotile nor motile *E. aerogenes* strains as part of the species *K. pneumoniae*. It has been suggested that, from data obtained by numerical taxonomy procedures, *E. aerogenes* strains be placed as a species within the genus *Klebsiella* (5). DNA relatedness data indicate that *E. aerogenes* is more closely related to *Klebsiella* species than to *E. cloacae* or other *Enterobacter* species (Brenner, *unpublished data*). The fact remains that relatedness among *Klebsiella* species is substantially greater than relatedness between *E. aerogenes* and any of the *Klebsiella* species. Among the alternatives available, one could retain these genera as presently constituted. The genus *Klebsiella* could be expanded to include *E. aerogenes* strains and possibly *E. cloacae* strains. In this case, the *E. hafniae* strains would probably not be included in the genus *Klebsiella*.

Ewing (16) stated that 95% of *Enterobacteriaceae* strains isolated from clinical sources can be identified by using the methods of biochemical differentiation worked out by him and his colleagues at the Center for Disease Control. Once an organism has been identified as a member of the tribe *Klebsielleae*, *E. aerogenes* can be differentiated from *Klebsiella* species based on indole, methyl red, Voges-Proskauer, citrate, urease, motility, and ornithine decarboxylase tests (17). The most difficult distinction occurs between *E. aerogenes* and *K. pneumoniae* where only motility, ornithine decarboxylase, and urease are useful. Several reports indicate that *K. pneumoniae* strains are sensitive to cephalosporins, whereas *E. aerogenes* strains are resistant to high concentrations of these antibiotics (7, 14, 21). This test is potentially of value in differentiating between *E. aerogenes* and klebsiellae, although the capacity of *Klebsiella* strains rapidly to acquire resistance to cephalosporins (7) and the generally shifting pattern of antibiotic resistance in strains isolated from clinical sources may limit its usefulness.

In an extensive study of organisms belonging to the tribe *Klebsielleae* (17), *K. pneumoniae* strains were shown to be 100% nonmotile, 95% urease negative, and 99% ornithine decarboxylase positive. No *Klebsiella* strains were both ornithine decarboxylase positive and urease negative, nor were any *E. aerogenes* strains both nonmotile and ornithine decarboxylase negative. Similar results have been obtained in other laboratories (3, 4, 14). Therefore, although the use of a single criterion for identification will be in error on occasion, the judicious use of all three criteria should prevent almost all misidentification. Strains atypical in other reactions that confuse identification can be differentiated by DNA relatedness. Since reassociation of DNA is not affected by point mutations or small deletions, we believe that DNA relatedness will distinguish between even the most atypical strains of *Klebsiella* and *E. aerogenes*.

Despite the fact that an average of greater than 40% of their genomes have diverged to a point where they cannot form stable duplexes, *K. pneumoniae* and *E. aerogenes* differ in very few biochemical reactions used in clinical differentiation. This seeming inconsistency is probably the result of three factors. (i) Different proteins can catatyze the same enzymatic reaction. It has been shown that *E. aerogenes* and *K. pneumoniae* contain diverged enzymes with a common function (2, 8). (ii) A given reaction end product may be produced from different pathways in different organisms. (iii) The number of biochemical tests employed by the most thorough clinical laboratory or numerical taxonomist rarely exceeds 150. The average bacterium contains enough DNA to specify some 3,000 genes, and

TABLE 4. *DNA relatedness among enteric bacteria*[a]

DNA reactions	Relative binding at 60 C (%)	Unpaired bases[b] within related sequences (%)	TBI[c]
Escherichia coli-Citrobacter strains	51	13	0.29
E. coli-Bethesda strains	44	13	0.19
E. coli-Salmonella spp.	45	14	0.19
E. coli-Klebsiella spp.	38	14	0.20
E. coli-Enterobacter spp.	40	13	0.17
E. aerogenes-Klebsiella spp.	56	9	0.71
E. aerogenes-E. cloacae	44	19	0.43
K. pneumoniae-E. cloacae	37	10	0.27

[a] *E. coli* reaction data from reference 10.
[b] Based on the assumption of 1% unpaired bases per degree decrease in duplex stability.
[c] TBI, Thermal binding index.

therefore the reactions employed test differences in only 5% of the total potential genetic information.

ACKNOWLEDGMENTS

We are indebted to W. H. Ewing, G. J. Hermann, and R. R. Colwell for providing most of the cultures used in this study.

LITERATURE CITED

1. Anderson, R. S., and E. J. Ordal. 1972. Deoxyribonucleic acid relationships among marine vibrios. J. Bacteriol. **109**:696-706.
2. Baptist, J. N., C. R. Shaw, and M. Mandel. 1969. Zone electrophoresis of enzymes in bacterial taxonomy. J. Bacteriol. **99**:180-188.
3. Barry, A. L., K. L. Bernsohn, and L. D. Thrupp. 1969. Rapid identification of *Escherichia, Klebsiella* and *Enterobacter* by use of a new urease test. Antimicrob. Ag. Chemother. 1968, p. 465-470.
4. Barry, A. L., K. L. Bernsohn, and L. D. Thrupp. 1969. Four-hour urease test for distinguishing between *Klebsiella* and *Enterobacter*. Appl. Microbiol. **18**:156-158.
5. Bascomb, S., S. P. Lapage, W. R. Willcox, and M. A. Curtis. 1971. Numerical classification of the tribe *Klebsielleae*. J. Gen. Microbiol. **66**:279-295.
6. Bautz, E. K. F., and F. A. Bautz. 1964. The influence of noncomplementary bases on the stability of ordered polynucleotides. Proc. Nat. Acad. Sci. U.S.A. **52**:1476-1481.
7. Benner, E. J., J. S. Micklewait, J. L. Brodie, and W. M. M. Kirby. 1965. Natural and acquired resistance of *Klebsiella-Aerobacter* to cephalothin and cephaloridine. Proc. Soc. Exp. Biol. Med. **119**:536-541.
8. Bowman, J. E., R. R. Brubaker, H. Frischer, and P. E. Carson. 1967. Characterization of enterobacteria by starch-gel electrophoresis of glucose-6-phosphate dehydrogenase and phosphogluconate dehydrogenase. J. Bacteriol. **94**:544-551.
9. Brenner, D. J., and S. Falkow. 1971. Molecular relationships among members of the *Enterobacteriaceae*. Advan. Genet. **16**:81-118.
10. Brenner, D. J., G. R. Fanning, K. E. Johnson, R. V. Citarella, and S. Falkow. 1969. Polynucleotide sequence relationships among members of *Enterobacteriaceae*. J. Bacteriol. **98**:637-650.
11. Brenner, D. J., G. R. Fanning, A. Rake, and K. E. Johnson. 1969. A batch procedure for thermal elution of DNA from hydroxyapatite. Anal. Biochem. **28**:447-459.
12. Brenner, D. J., G. R. Fanning, F. J. Skerman, and S. Falkow. 1972. Polynucleotide sequence divergence among strains of *Escherichia coli* and closely related organisms. J. Bacteriol. **109**:953-965.
13. Britten, R. J., and D. E. Kohne. 1966. Nucleotide sequence repetition in DNA. Carnegie Inst. Washington Yearb. **65**:78-106.
14. Edmondson, E. B., and J. P. Sanford. 1967. The *Klebsiella-Enterobacter (Aerobacter)-Serratia* group: a clinical and bacteriological evaluation. Medicine (Baltimore) **46**:323-340.
15. Eichhoff, T. C., B. W. Steinhauer, and M. Findland. 1966. The *Klebsiella-Enterobacter-Serratia* division: biochemical and serological characteristics and susceptibility to antibiotics. Ann. Intern. Med. **65**:1163-1179.
16. Ewing, W. H. 1970. Differentiation of *Enterobacteriaceae* by biochemical reactions. Center for Disease Control, Atlanta, Ga.
17. Fife, M. A., W. H. Ewing, and B. R. Davis. 1965. The biochemical reactions of the tribe *Klebsielleae*. Center for Disease Control, Atlanta, Ga.
18. Graber, C. D. 1970. The *Enterobacteriaceae*, p. 30-47. *In* C. D. Graber (ed.), Rapid diagnostic methods in medical microbiology. The Williams & Wilkins Co., Baltimore.
19. Kingsbury, D. T., G. R. Fanning, K. E. Johnson, and D. J. Brenner. 1969. Thermal stability of interspecies *Neisseria* DNA duplexes. J. Gen. Microbiol. **55**:201-208.
20. Laired, C. D., B. L. McConaughy, and B. J. McCarthy. 1969. On the rate of fixation of nucleotide substitutions in evolution. Nature (London) **224**:149-154.
21. Ramirez, M. J. 1968. Differentiation of *Klebsiella-Enterobacter (Aerobacter)-Serratia* by biochemical tests and antibiotic susceptibility. Appl. Microbiol. **16**:1548-1550.