JOURNAL OF BACTERIOLOGY, Nov. 1968, p. 1783-1790

Vol. 96, No. 5
Printed in U.S.A.

Homologies of Deoxyribonucleic Acids from *Brucella ovis*, Canine Abortion Organisms, and other *Brucella* Species

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Received for publication 13 August 1968

The bacterium that causes canine abortion has polynucleotide sequences similar, in deoxyribonucleic acid (DNA)-DNA homology studies, to those of *Brucella suis* and, by inference from previous data, those of *B. abortus* and *B. melitensis* as well as *B. neotomae*. Therefore, the organism causing canine abortion appears to be a member of the genus *Brucella*. DNA preparations from *Serratia marcescens*, *Alcaligenes faecalis*, and *Bordetella bronchiseptica*, 58, 62, and 66 mole % guanine plus cytosine, respectively, do not have detectable polynucleotide sequence homologies with *B. suis* DNA which is 56 mole % guanine plus cytosine. *B. ovis* DNA lacks some of the polynucleotide sequences present in *B. suis* DNA and appears to be a deletion mutant. However, a large proportion of *B. ovis* polynucleotides are similar to those of other *Brucella* species, which supports the inclusion of *B. ovis* in the genus.

Studies of deoxyribonucleic acid (DNA) polynucleotide sequence homologies within the genus *Brucella* were presented in a previous report (8). In reciprocal DNA-agar competition reactions performed at 60 C in 2 × SSC (SSC is 0.15 M sodium chloride plus 0.015 sodium citrate), the polynucleotide sequences of the DNA of type I of each of the classical (principal) species, *B. abortus*, *B. suis*, and *B. melitensis*, were similar. These competition reactions did not distinguish among the species. The DNA from *B. neotomae* (15) was as effective a competitor as that from any of the principal species, whereas the DNA from *B. ovis* (2) was less effective.

In 1966, a brucella-like organism was isolated from an aborting beagle by Carmichael (3) and was established as the cause of epidemic infectious abortion in dogs (L. E. Carmichael and D. W. Bruner, Cornell Vet., *in press*). Although resembling the members of the genus *Brucella* in many respects, this organism, like *B. ovis*, lacks surface antigens present in smooth cultures of the principal *Brucella* species. Classical approaches failed to clearly establish the taxonomic relationships of this new organism. We have, therefore, examined the polynucleotide sequence relationships of the beagle organism DNA and *B. suis* DNA. These sequences are similar enough to support inclusion of the beagle organism in the genus *Brucella*.

Also, more detailed studies of the relationship of *B. ovis* to other members of the genus have disclosed that *B. ovis* lacks a portion of the polynucleotide sequences shared by other members of the genus.

MATERIALS AND METHODS

Microorganisms. Bacterial cultures used for the preparation of radiolabeled and unlabeled DNA were *B. suis* 1776, *B. ovis* 232, *B. neotomae*, and the organism of canine infectious abortion (RM-666). This latter culture was supplied by L. E. Carmichael of Cornell University, Ithaca, N.Y., and had been isolated from a beagle bitch during an epidemic of infectious abortion. This organism, called the beagle organism, resembles the members of the genus *Brucella* in many respects, but it lacks antigenic relationship with smooth *Brucella* cultures when tested for reciprocal agglutination. A relationship with the deeper cell antigens of the principal species has been demonstrated (5).

In addition, unlabeled DNA was prepared from typical strains of *Alcaligenes faecalis*, *Bordetella bronchiseptica*, and *Serratia marcescens*.

All cultures were examined for adherence to species characteristics prior to use. Final suspensions of cells used for preparation of DNA were examined for purity by observation of stained smears and colonial morphology of subcultures.

Media and conditions of growth. For unlabeled DNA, all organisms were grown on Trypticase Soy Agar (BBL). Agar medium in large culture flasks was inoculated with 24-hr slant cultures of the various organisms. Cultures of *B. ovis* were placed in an atmosphere containing 10% added CO_2. All cultures were incubated at 37 C: *A. faecalis*, *B. bronchiseptica*, and *S. marcescens* for 24 hr, and the others for 48 to

72 hr. The cells were harvested by removing the surface growth with phosphate-buffered saline.

For preparation of ^{32}P-labeled DNA, low phosphate was necessary. An agar medium was prepared with the two peptones present in Trypticase Soy Agar that had the following composition: Trypticase 1.7%, Phytone 0.3%, sodium chloride 0.5%, dextrose 0.25%, and agar 2%. The *p*H was adjusted to 7.0. The medium was dispensed in large culture bottles, 70 ml per bottle. To each bottle was added 5 mc of carrier-free ^{32}P-orthophosphate. The complete medium was sterilized at 120 C for 30 min. Agar-layered bottles were seeded with bacteria from 24-hr slants and were incubated at 37 C for 72 hr (*B. ovis* in 10% CO_2 plus air). The cells were harvested by removing the surface growth with phosphate-buffered saline. For some experiments, highly labeled DNA was required. In these instances, 18 mc of carrier-free ^{32}P-orthophosphate was added per culture bottle, and the amount of agar was reduced to 40 ml to promote uptake.

Preparation of high molecular weight DNA. DNA was prepared as previously described (1, 8). However, "spooled," resuspended, high molecular weight DNA was treated with 50 μg of pancreatic ribonuclease per ml at 60 C for 1 hr in one-tenth strength SSC, followed by incubation with 50 μg of Pronase per ml (Calbiochem, Los Angeles, Calif.) in 0.02 M tris(hydroxy-methyl)aminomethane-(Tris)-chloride buffer (*p*H 8.2) and 5 mg of sodium lauryl sulfate (SLS) per ml at 37 C overnight. Pronase (500 μg/ml) was "self-digested" in 0.02 M Tris-chloride (*p*H 8.2) and 5 mg of SLS per ml for 1 hr at 37 C prior to use. The SLS concentration was then raised to 10 mg/ml, sufficient NaCl to make the solution 0.1 M, and an equal volume of water-washed phenol was added. This mixture was shaken for 15 min under conditions just adequate to maintain the phases thoroughly mixed. The aqueous phase was separated by centrifugation at 17,500 × *g* for 30 min, removed, mixed with two volumes of ethyl alcohol, and again "spooled." Spooled DNA on a glass rod was washed with sufficient 0.1 M NaCl plus two volumes of ethyl alcohol to remove traces of phenol. The DNA was then stored in screw-cap vials containing one-tenth strength SSC and 0.5 ml of chloroform.

Preparation of DNA fragments. Radiolabeled or unlabeled higher molecular weight DNA prepared as indicated above was sheared and purified as previously described (8). If necessary, the DNA fragments in 0.1 M NaCl were concentrated by precipitation with three volumes of cold ethyl alcohol and dialyzed against one-tenth strength SSC.

Preparation of DNA filters. High molecular weight native DNA, about 200 μg/ml in 25 ml, was denatured by 0.30 N NaOH at room temperature for 10 min. The mixture was cooled to 0 C in an ice bath, 0.5 ml of 1 M Tris-chloride (*p*H 8.2) was added and, finally, sufficient 1.00 N HCl was added to neutralize the NaOH. The neutralized mixture was centrifuged at 17,500 × *g* for 30 min at 0 C. Any pellet was discarded, and the supernatant fluid was diluted to 500 ml in ice-cold 4× SSC. The DNA solution at 4 C was then slowly passed through a B-6 Schleicher & Schuell Co. (Keene, N.H.) membrane filter (14 cm in diameter) which had been previously incubated 4 hr at 68 C in 4× SSC and subsequently washed with 1 liter of 4× SSC at about 80 C. After application of the DNA, the filter was rapidly washed with 1 liter of 4× SSC at 4 C. The amount of DNA fixed to the filter was determined by monitoring the optical density, at 259 nm, of the input and the washes. The filter was removed, placed at room temperature under flowing air for 4 to 6 hr, and dried in an oven (60 C) for 12 to 16 hr. Circles (2 cm in diameter) were cut from the large filter with a sharpened cork borer. These filters were stored in a sealed container at −20 C until used.

Prior to use, the filters were incubated at 68 C for 3 to 4 hr in 4× SSC containing the preincubation mixture (PM) of Denhardt (4).

DNA-DNA reassociation and competition reactions. To a scintillation vial, placed in crushed ice, 0.250 ml of 8× SSC with double-strength PM and 0.250 ml of one-tenth strength SSC, with or without melted, unlabeled DNA fragments were added. A 1-μg amount of homologous, melted, ^{32}P-labeled DNA fragments in 0.025 ml one-tenth strength SSC was added to each vessel, and a blotted, preincubated DNA-nitrocellulose filter (2 cm in diameter) was introduced. The vial was sealed with a Teflon-lined screw cap, and air bubbles were removed from the liquid which barely covered the filter. The vials were placed in a 68 C water bath for 16 hr.

Upon completion of the incubation, the reaction liquid was removed, and the filter was washed four times with 5 ml of 4× SSC. Each wash was removed with a capillary pipette and added to the tube which contained the original reaction liquid. The DNA in the combined washes was precipitated with 5% trichloroacetic acid, collected on a nitrocellulose filter, and counted in a Packard scintillation spectrometer to determine the amount of unbound, radiolabeled DNA.

The washed filter was wrapped in a square of Saran mesh and placed, filter toward the axis, in an open glass tube (10 by 200 mm). These tubes were placed in test tubes (16 by 150 mm), each containing 20 ml of 4× SSC with PM at 70 C (wash solution). At 10- to 15-min intervals, the filters were transferred to tubes containing fresh wash solution. These washes contained only 1 to 2% of the unbound counts, and they were discarded. After four transfers, the filters were blotted and dried at 60 C for 30 min. The dried filters were supported in a diagonal position, by a straight pin, in scintillation vials containing 15 ml of scintillation fluid; they were counted in a Packard scintillation spectrometer.

In some experiments, the bound DNA was recovered for reuse. In those instances, filters (washed as above) were finally placed for 10 min in a test tube containing 5 ml of distilled water at 90 C. The filter was removed, the NaCl concentration was adjusted to 0.1 M, 500 μg of purified yeast ribonucleic acid (RNA) was added, and the DNA was precipitated with three volumes of ethyl alcohol at −20 C overnight and was centrifuged. The precipitate was dissolved in a minimal volume of distilled water and dialyzed against one-tenth strength SSC.

Fractionation of ^{32}P-labeled DNA. DNA was fractionated by thermal elution from hydroxylapatite, essentially as described by Miyazawa and Thomas (14). Sheared, ^{32}P-labeled *B. suis* DNA fragments in 0.03 M K_2HPO_4-KH_2PO_4 (PB) were adsorbed to a column (10 by 30 mm) of Hypatite-C (Clarkson Chemical Co., Williamsport, Pa.) at 60 C, in a jacketed container. The PB concentration was raised to 0.08 M, and the temperature was elevated in desired increments. At each temperature, 0.08 M PB was pumped through the column at about 20 ml per 5 min. Either two or three 20-ml fractions were collected at each temperature in two separate experiments. Effluents were counted directly in a Packard liquid scintillation spectrometer by means of Cerenkov radiation. Eluted fractions were precipitated by the addition of three volumes of ethyl alcohol in the presence of 500 μg of purified yeast RNA, and the precipitates were dialyzed against 0.1 M NaCl plus 0.01 M ethylenediaminetetraacetate (pH 8.0), and, finally, against one-tenth strength SSC.

"Melting" of ^{32}P-labeled DNA fragments from nitrocellulose filters. After incubation and washing at 70 C, as indicated above, reassociated, radiolabeled DNA fragments were melted from DNA fixed on nitrocellulose filters in 4× SSC essentially as described by McCarthy (13) and by Johnson and Ordal (9).

Determination of base composition of DNA. The midpoints of the melting temperatures of the DNA were determined, and the guanine plus cytosine (GC) contents were calculated as previously described (8). *Escherichia coli* DNA, dialyzed together with test DNA, was used as a reference.

RESULTS AND DISCUSSION

Base compositions of the DNA preparations were determined (Table 1) from the midpoints of their melting curves (T_m) in SSC or 0.25 SSC at 259 nm, as described by Marmur and Doty (11). Beagle organism DNA has a GC content (56 mole %) very similar to that of *B. neotomae*. It was previously determined that the base composition of *B. neotomae* DNA is the same as the classical species DNA and *B. ovis* DNA (8).

Historically, *Brucella* organisms have been grouped with several other genera which include *Alcaligenes* and the present *Bordetella*. Therefore, DNA preparations from *A. faecalis* and *B. bronchiseptica* (62 and 66 mole % GC, respectively) were included in these studies. *S. marcescens* DNA (58 mole % GC) was also included as a control for the specificity of the homology determinations, because its base composition and that of *Brucella* are similar. The base compositions determined for *A. faecalis* and *S. marcescens* are in agreement with those previously reported (7), whereas no record of that for *B. bronchiseptica* was found.

Polynucleotide sequence similarities of beagle organism and principal species of Brucella. Because the principal species have equivalent DNA homologies, in reciprocal tests, it is assumed that one representative, *B. suis*, may be used as the indicator for all three. Therefore, high-molecular weight, denatured *B. suis* DNA was fixed to nitrocellulose filters (6) and reacted with ^{32}P-labeled, melted *B. suis* DNA fragments in the presence or absence of unlabeled, melted DNA fragments (competitor) from *B. suis* or heterologous bacteria. Unlabeled beagle organism DNA fragments compete as effectively as *B. suis* fragments (Fig. 1a). Also, *B. ovis* fragments compete nearly as well as those from *B. suis* and the beagle organism but, as previously reported (8), are consistently less effective. Thus, results obtained with DNA fixed to membrane filters incubated overnight at 68 C in 4× SSC plus PM are in good agreement with those obtained with DNA-agar incubated overnight at 60 C in 2× SSC. Nonspecific binding of ^{32}P-labeled DNA fragments to blank filters or *E. coli* DNA filters was 0.01 to 0.05% of the input counts.

Reciprocal competition reactions of beagle organism fixed DNA with homologous, radiolabeled beagle organism DNA fragments (Fig. 1c) agreed with those obtained with *B. suis* DNA. Also, Beagle organism and *B. suis* DNA fragments compete as effectively as fragments from *B. ovis* in the homologous *B. ovis* system (Fig. 1b). It may be concluded, from three independent observations, that beagle organism DNA has polynucleotide sequences which are indistinguishable from those of *B. suis* (and, by inference, the other two principal species) and is, by DNA-DNA homologies, a member of the genus *Brucella*.

Specificity of the competition reactions. DNA fragments from *A. faecalis*, *B. bronchiseptica*, and

TABLE 1. *Base composition of the DNA preparations from the test organisms*[a]

T_m in	Beagle organism	*B. neotomae*	*E. coli*	*A. faecalis*	*B. bronchiseptica*	*S. marcescens*
SSC	92.2	92.0	89.7		96.2	93.2
0.25 × SSC			82.3	87.3	88.9	
Mole % GC	56	56	50	62	66	58

[a] Calculations and designations are the same as those made previously (8).

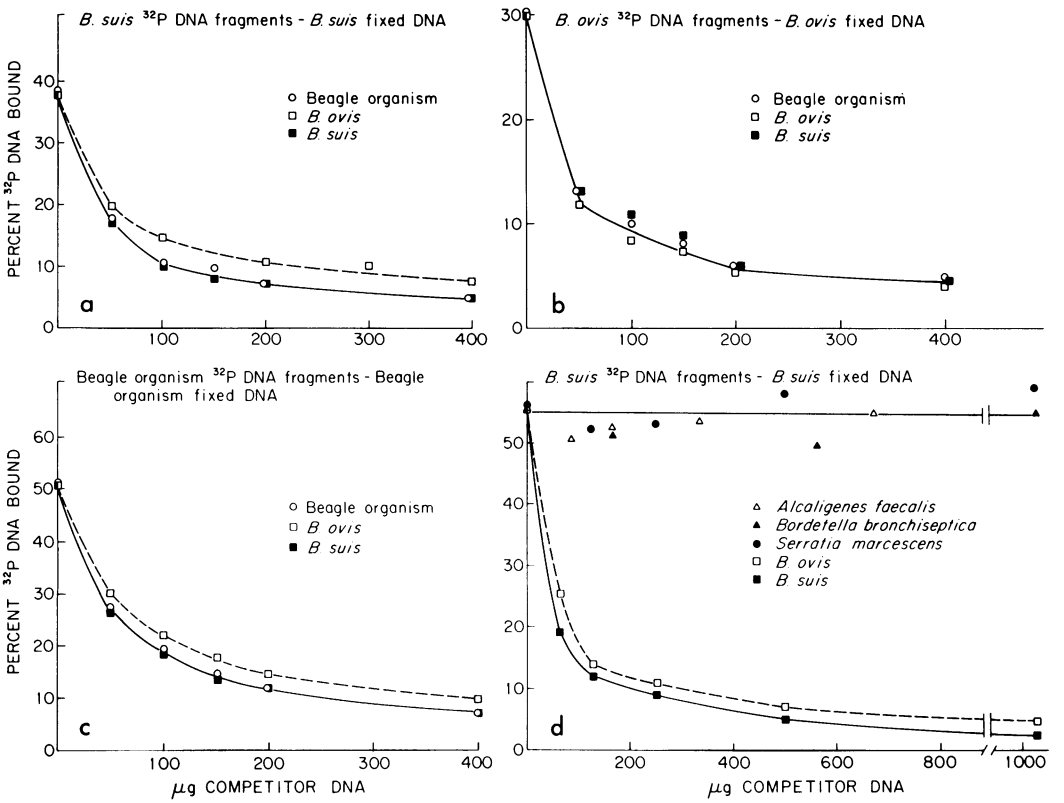

FIG. 1. *A 1-μg amount of 32P-labeled, sheared, melted DNA fragments was incubated with homologous DNA fixed to nitrocellulose filters. Unlabeled, sheared, melted DNA fragments were used as competitors in the amounts indicated. Filters each contained an average of 34, 55, 51, and 80 μg of fixed DNA (Fig. 1a through 1d), respectively. 32P-labeled DNA fragments used had specific activities (counts per min per μg) of 73 × 10³, 50 × 10³, 67 × 10³, and 185 × 10³ (Fig. 1a through 1d), respectively.*

S. marcescens are not competitors in the *B. suis* reassociation reaction (Fig. 1d); these DNA preparations have average GC contents (62, 66 and 58 mole %, respectively) approximately equal to or greater than those of *Brucella* species. Their lack of competitive effect indicates that the reaction conditions were sufficiently stringent to prevent adventitious reactions such as those described for the high GC DNA of pseudomonads and myxobacteria (9). As previously noted (8), there is detectable scatter in the results obtained with noncompeting DNA preparations, but there is no consistent trend indicating a degree of polynucleotide sequence homology.

Detection of deletions in the B. ovis genome. Figures 1a, 1c, 1d, and previous observations (8) all indicate that *B. ovis* DNA fragments do not compete as effectively as DNA fragments from other *Brucella* species when fixed DNA from these other species is incubated with homologous, radiolabeled DNA fragments in the presence of the competitors. These findings indicate that *B. ovis* does not contain all of the polynucleotide sequences shared by the DNA of other *Brucella* species. However, the reaction of 32P-labeled *B. ovis* DNA fragments with *B. ovis* DNA on nitrocellulose filters is decreased to the same extent by unlabeled beagle organism, *B. suis*, and *B. ovis* DNA fragments. This seeming discrepancy can be satisfactorily explained by the assumption that the *B. ovis* DNA has undergone a deletion or series of deletions so that a detectable proportion of its DNA is now missing when compared to the DNA of other *Brucella* species.

The presence of a detectable proportion of DNA unique to *B. ovis*, in addition to that common to other *Brucella* species, is not supported by our evidence. If unique DNA were present in *B. ovis*, beagle organism and *B. suis* DNA fragments should not be as effective as competitors as the homologous *B. ovis* fragments in the system (Fig. 1b). In contrast, Fig. 1b indicates that all three

competitors are equally effective. If unique DNA, in addition to a high proportion of common DNA, were present, the results would be similar to those of Fig. 1a. This is clearly not the case.

The polynucleotide homologies of *B. ovis* and *B. suis* were examined in three ways. First, the stability of the homologous and heterologous reaction products between these two species was examined; second, an enrichment of the sequences unique to *B. suis* in the pair was attempted; and third, the *B. suis* DNA was fractionated on the basis of its base composition distribution (12, 14) to enable detection of a possibly different GC content of the unique sequences.

Thermal stability of B. ovis and B. suis reassociation products. Nitrocellulose filters with fixed *B. ovis* or *B. suis* DNA were incubated overnight at 68 C in 4× SSC with either *B. ovis* or *B. suis* ^{32}P-labeled, melted DNA fragments. Thus, all combinations were represented. The incubated filters were washed at 70 C in 4× SSC. Each filter was then placed in a tube containing 10 ml of 4× SSC, maintained for 5 min at the desired temperature, and transferred to a fresh tube of 4× SSC. The temperature was raised in 3 to 4 C increments from 70 to 100 C. At each temperature, therefore, radiolabeled DNA released from the DNA on the filter remained in the 4× SSC. This procedure is essentially that described by McCarthy and coworkers (13). Carrier RNA was added to each tube, and the ^{32}P-labeled DNA was precipitated at 0 C by 5% trichloroacetic acid. The precipitates were collected on membrane filters, and their radioactivity was determined. Integral "melting" curves were constructed, and the midpoint of these curves (analogous to the T_m) was determined. The midpoint of the melting curves of either *B. ovis* or *B. suis* DNA fragments from *B. ovis* DNA filters was 88 C. The radioactive fragments were released from the DNA on the filter from 74 to 98 C. With *B. suis* DNA filters, the radioactive fragments were released from 74 to 99 C, and the midpoint was 89 C for *B. suis* and 90 C for *B. ovis* DNA fragments. Separate determinations of loss of radiolabeled, fixed DNA from the filters indicated that 25 to 30% of the fixed DNA was freed during the elution procedure. The loss of fixed DNA from the filters probably accounts for the low (7 to 9 C below the expected T_m) melting temperatures observed. However, these results indicate no appreciable differences in the thermostability of the homologous and heterologous reassociation products.

Enrichment of the DNA sequences of B. suis which are not common to B. ovis. Differences between the *B. suis* and *B. ovis* polynucleotide sequences, as indicated by competition experiments, are small but consistent (Fig. 1a, 1c, 1d). However, these differences should be accentuated by successive cycles of selection for the sequences unique to *B. suis*. To accomplish such selection, *B. suis* DNA fragments of high specific radioactivity were reacted with fixed *B. suis* DNA in the presence of 400 μg of unlabeled *B. ovis* DNA fragments. The radiolabeled DNA reassociated with the fixed DNA should, therefore, be enriched for sequences unique to *B. suis* DNA. This enriched DNA was recovered and again reacted with *B. suis* DNA filters in the presence of 400 μg of unlabeled *B. ovis* DNA fragments. Table 2 indicates the results of such a two-step enrichment. With unselected ^{32}P-labeled *B. suis* DNA fragments, the results are in good agreement with those indicated in Fig. 1. *B. suis* competitor reduces the binding of *B. suis* radiolabeled DNA to *B. ovis* or *B. suis* DNA filters to about the same extent; in contrast, *B. ovis* competitor is less effective with *B. suis* DNA filters than with *B. ovis* filters. Selected, radiolabeled *B. suis* fragments associate with either *B. ovis* or *B. suis* fixed DNA about the same as unselected fragments in the absence of competitor DNAs; there is some indication of a selection, since the binding to *B. ovis* DNA filters was reduced from 26 to 22%, whereas the binding to *B. suis* filters increased. However, *B. ovis* com-

TABLE 2. *Enrichment of polynucleotide sequences of B. suis DNA which are not shared by B. ovis DNA*

B. suis fragments[a]	Source of unlabeled competitor DNA	*B. suis* ^{32}P-DNA fragments bound (%) to DNA filter with	
		B. ovis DNA	*B. suis* DNA
Unselected	None	26	37
	B. ovis	4	7
	B. suis	3	4
Selected	None	22	41
	B. ovis	3	13
	B. suis	3	4

[a] ^{32}P-labeled, melted, sheared *B. suis* DNA fragments (2 μg; 35,000 counts per min per μg) were incubated with *B. ovis* or *B. suis* DNA filters which contained 55 or 34 μg of DNA, respectively, per filter. In one set of incubations, no competitor DNA was added, and in another, 400 μg of either *B. ovis* or *B. suis* unlabeled, sheared, melted DNA fragments was included. Selected, ^{32}P-labeled DNA was recovered from a duplicate *B. suis* DNA filter incubated as above with 400 μg of *B. ovis* DNA competitor. These recovered, selected fragments (about 0.01 μg per incubation) were again melted and reacted with *B. ovis* or *B. suis* DNA filters in the presence or absence of the two competitor DNA preparations (400 μg).

petitor does not reduce the binding of selected *B. suis* DNA fragments to *B. suis* fixed DNA as effectively as it reduces the binding of unselected fragments; *B. ovis* competitor was $1.00 - (7 - 4:37 - 4) \times 100 = 91\%$ as effective as *B. suis* competitor with the unselected *B. suis* labeled fragments and $1.00 - (13 - 4:41 - 4) \times 100 = 76\%$ effective with the selected fragments. This represents a 2.7-fold change in relative efficiency of the *B. ovis* competitor when assayed with selected: unselected *B. suis* fragments on *B. suis* fixed DNA. One additional cycle of selection was performed, and a further twofold enrichment was observed.

No enrichment was noted when a similar series of experiments were carried out with *B. ovis* or *B. suis* competitors in the *B. ovis* reassociation system, nor was enrichment observed when *B. suis* competitor was used in the *B. suis* system.

Therefore, an effective enrichment of the proposed unique *B. suis* sequences was demonstrated. This supports the proposal that the *B. ovis* genome developed by deletion of DNA sequences of one of the principal species.

Competition between B. ovis DNA and fractionated B. suis DNA. Miyazawa and Thomas (14) demonstrated that hydroxylapatite columns were effective in fractionating DNA fragments on the basis of their GC contents. This fractionation is based on the observation that double-stranded DNA is retained on hydroxylapatite in 0.08 M phosphate buffer, whereas single-stranded DNA is not. Therefore, if the buffer is held constant and the temperature gradually increased, those fragments which melt are eluted. Also, it has been demonstrated that small DNA fragments of bacteriophages (14) and higher organisms (12) have a range of GC compositions which differ from the average value.

The DNA segments of *B. suis*, not present in *B. ovis*, could have a unique GC distribution. If so, a marked enrichment of the uncommon sequences would be expected in the portions of the eluates from a hydroxylapatite column which have the composition representative of the unique fraction. Figure 2a indicates the elution pattern of *B. suis* double-stranded DNA fragments. Figure 2b is a melting curve constructed from a plot of the cumulative counts versus temperature. A duplicate elution, with only two fractions per temperature, gave nearly identical results.

As previously demonstrated (12, 14), those fragments low in GC elute at relatively low temperatures and those high in GC elute at relatively high temperatures. Three fractions were chosen for further examination (Fig. 2). The ^{32}P-labeled *B. suis* DNA fragments in these fractions were precipitated and dialyzed.

All three fractions reacted with *B. suis* DNA on nitrocellulose filters to about the same extent (Table 3). All three, however, reacted somewhat less well than unfractionated DNA. A possible explanation of this result is that all three fractions, because they represent only specific portions of the whole DNA, are present in appreciably higher concentration than the whole DNA and are partially removed from reaction with the filter DNA by reassociation in free solution.

B. ovis DNA is a consistently less effective competitor than the homologous DNA for all fractions. There is, however, an apparent enrichment for sequences predominantly resident in *B. suis* DNA in the low-temperature fraction. This fraction is 1.35-fold less effectively competed with (Table 3) by the *B. ovis* DNA fragments. This result may possibly be caused by the relatively more stringent incubation conditions imposed on the low GC, fraction 1, sequences or to the uncertainty introduced by the rather low binding. A proportion of the polynucleotide sequences found in *B. suis* but not *B. ovis* DNA may, thus, be relatively low in GC as compared with the total DNA. However, it is evident that all of the fractions examined contain sequences less effectively competed with by *B. ovis* than *B. suis* DNA fragments. It may be concluded that the polynucleotide sequences found in *B. suis* but not *B. ovis* are very likely present throughout its genome, and that it is unlikely that *B. ovis* arose by a single deletion but, rather, by multiple small deletions.

General considerations of the data presented. Bacteria classified in the genus *Brucella* by biochemical, immunological, and pathological behavior also constitute a very homogeneous group when examined for the ability of their polynucleotide sequences to interfere with the reassociation of radiolabeled DNA fragments with homologous single-stranded DNA on nitrocellulose filters or in DNA-agar (8). The beagle organism lacks antigens present in smooth cultures of the classical *Brucella* species, and because of this phenomenon was not readily recognized as a member of the genus. However, in our homology studies, its DNA was indistinguishable from that of *B. suis* (and, by inference, the other classical species). This finding clearly denotes the taxonomic position of this organism and warrants its acceptance as a member of the genus *Brucella* (*B. canis*), as suggested by L. E. Carmichael and D. W. Bruner (*in press*). *B. ovis* DNA is preponderantly similar to the DNA of other *Brucella* by DNA homology studies. Therefore, inclusion of this organism in the genus appears justified.

Conservation of polynucleotide sequences sufficiently similar to be indistinguishable by DNA-DNA competitions appears to be a re-

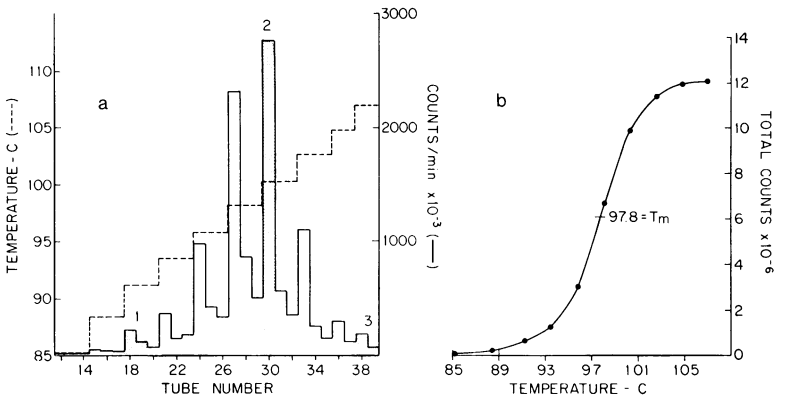

FIG. 2. *A 22-μg amount of B. suis native ^{32}P-labeled DNA fragments (300,000 counts per min per μg) was loaded at 60 C onto a Hypatite-C column (1 by 3 cm) in 0.03 M KH_2PO_4-K_2HPO_4. The column was washed with 120 ml of 0.03 M buffer followed by 0.08 M buffer at 60 C. The temperature was raised gradually to 85 C at a buffer flow of about 4 ml per min. The temperature was raised stepwise as indicated and, when the temperature had equilibrated, three separate 20-ml fractions were collected at each temperature. Of the counts added to the column, 87% were recovered in the portions of the elution profile depicted. Numbers 1, 2, and 3 indicate the fractions referred to in Table 3. The right side of the figure is a plot of the cumulative counts eluted versus temperature. The high T_m indicated is accounted for by the fact that T_m values determined from hydroxylapatite elutions are slightly higher than those determined spectrophotometrically (14), and the stepwise temperature increments were about 5 C.*

TABLE 3. *Unlabeled B. ovis and B. suis DNA fragments as competitors in the binding of hydroxylapatite-temperature-fractionated ^{32}P-labeled B. suis DNA fragments to fixed B. suis DNA*

Labeled *B. suis* DNA[a]	Labeled fragments bound (%) in presence of			Effectiveness of *B. ovis*[b]	Difference factor[c]
	No competitor	*B. suis* DNA fragments (1000 μg)	*B. ovis* DNA fragments (1000 μg)		
				%	
Unfractionated	61.7	2.2	4.4	96.3	
Low temperature (1)	55.5	3.2	7.0	92.7	1.35
Mid-temperature (2)	57.0	2.4	5.3	94.7	1.00
High temperature (3)	53.5	3.0	5.8	94.5	1.00

[a] About 0.02 μg of *B. suis* ^{32}P-labeled DNA fragments (32,000 counts per min per μg) in 4× SSC was incubated, 68 C, overnight with nitrocellulose filters containing 80 μg of *B. suis* DNA. Numbers in parentheses indicate the hydroxylapatite prepared fractions designated in Fig. 2.

[b] Per cent effectiveness was calculated as follows: unfractionated, 1.00 − (4.4 − 2.2:61.7 − 2.2) × 100 = 96.3%; low temperature (1), 1.00 − (7.0 − 3.2:55.5 − 3.2) × 100 = 92.7%; mid-temperature (2), 1.00 − (5.3 − 2.4:57.0 − 2.4) × 100 = 94.7%; high temperature (3), 1.00 − (5.8 − 3.0:53.5 − 3.0) × 100 = 94.5%.

[c] The difference factor was calculated as follows: fractions (2) and (3) were considered equivalent and averaged (94.7 + 94.5:2) = 94.6% or 100 − 94.6 = 5.4% less effective than *B. suis* fragments; fraction (1) is thus 7.3% less effective; 7.3/5.4 = 1.35, the difference factor.

markable property of the members of the genus *Brucella*. The genus *Brucella* contains representatives which are of worldwide distribution and have widely divergent host ranges. An interesting speculation may be that groups of parasitic, particularly intracellular, bacteria may tend to be more homogeneous than groups of free-living organisms because their maintenance in nature is partially determined by the restrictive nature of the parasitic or intracellular environment. Data obtained by Kingsbury (10) with the *Neisseria* seem to support this speculation.

Some studies of DNA-DNA relationships of groups of organisms have apparently been misleading because of the use of criteria not capable of being sufficiently discriminate; a striking ex-

ample (9) was the inability to differentiate pseudomonads and myxobacteria, both groups having DNA of high mole % GC, under the generally employed incubation conditions. It is unlikely that these studies of the *Brucella* have suffered from such difficulties because DNA from organisms with GC contents equivalent to, and higher than, those of the *Brucella* species did not compete in the *Brucella* DNA-DNA associations.

It must, however, be reemphasized that DNA-DNA homology studies are not as discriminatory as studies of infectious processes, immunological properties, and biochemical and enzymologic reactions. Now the DNA-DNA studies, along with more classical approaches, are capable of assigning newly discovered organisms to a logical position within existing groups. They are not yet capable, however, of detecting fine differences.

Nevertheless, DNA-DNA competition reactions were able to detect the relatively small proportion of sequences deleted from *B. ovis* DNA and to indicate that the altered host range and immunological properties of this organism are probably the result of deletions which occurred in nature. The existence of these deletions may make *B. ovis* an ideal recipient of DNA from other *Brucella* species in transformation studies.

ACKNOWLEDGMENT

The excellent technical assistance of William G. Woodend has been of great value.

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