Deoxyribonucleic Acid Homology of *Aerococcus viridans*¹

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Deoxyribonucleic acid (DNA) was purified from cultures of 16 strains that, on the basis of physiological tests, appeared to be strains of *Aerococcus viridans* (although one was named *Gaffkya homari* and two were called *Aerococcus catalasicus*). The DNA base composition of 15 of these strains, as determined by the thermal denaturation technique, was within the range of 37.0 to 40.2% guanine plus cytosine. The relative nucleotide sequence homology was measured by DNA-DNA hybridization on nitrocellulose membrane filters. The DNA from most strains hybridized with the DNA from other strains to the extent of 69 to 100% of the level of homologous binding. Limited studies indicated that under the conditions of these studies there was less than 12% homology with the DNA of several pediococci, one enterococcus, and one strain of *Gaffkya tetragena*.

The bacterial species *Aerococcus viridans* (9) appears to be widely dispersed in our environment and to be quite homogeneous with respect to physiological characteristics, nutritional requirements, and deoxyribonucleic acid (DNA) base composition (4, 5, 8). However, there remain questions regarding its taxonomic relationship to other genera of gram-positive cocci, particularly those with a tendency toward tetrad formation and with a DNA base composition between 30 and 45% guanine plus cytosine (GC). The present study was an attempt to learn more about the nucleotide homology of these organisms by measuring the relative amount of DNA-DNA hybrid formation under standardized conditions.

MATERIALS AND METHODS

Cultures and growth conditions. The bacterial strains studied and their sources are listed in Table 1. Cells for DNA extraction were grown at 30 C in Trypticase Soy Broth (BBL). Cells were harvested in the early stationary phase. If the test culture was a pediococcus or a streptococcus, 1,000 units of penicillin G (Eli Lilly & Co., Indianapolis, Ind.) was added

to the flask 4 to 6 hr before harvesting the cells. This treatment rendered the cells sensitive to lysozyme.

Production of labeled DNA. Most cultures were labeled by the addition of 15 μ Ci of adenine-8-¹⁴C (Calbiochem, Los Angeles, Calif.) per liter of growth medium. However, some cultures did not incorporate adenine; for these, 20 μ Ci of [methyl-³H] thymidine (New England Nuclear, Boston, Mass.) was added per liter of medium.

DNA isolation. The harvested cells were lysed, and the DNA was extracted and purified by the method of Marmur (6) as modified by Auletta and Kennedy (1). The amount of DNA was measured as absorbance at 260 nm.

Determination of DNA base composition. Thermal denaturation curves were determined, and the DNA base composition as moles per cent guanine plus cytosine (% GC) was calculated by the method of Marmur and Doty (7).

DNA-DNA hybridization. Unlabeled DNA was denatured by heating at 100 C for 5 min in SSC (0.15 M NaCl and 0.015 M sodium citrate, pH 7.0). Twenty-microgram amounts of this DNA in 6 $\times$ SSC were fixed on 25-mm nitrocellulose membrane filters (B-6, Schleicher and Schuell Co., Keene, N. H.) by the method of Denhardt (3). Filter quarters with DNA affixed were placed in scintillation vials with 1.0 ml of Denhardt's preincubation mix (all ingredients from Sigma Chemical Co., St. Louis, Mo.) at 67 to 69 C for 5 to 6 hr. Labeled DNA in SSC was sheared by passing 10 times through a 1-ml tuberculin syringe with a 5/8-inch 25-gauge needle, denatured, cooled, and brought to 2 $\times$ SSC. A 0.5-ml amount (1 to 5 μ g) was added to a filter quarter that had been removed from the preincubation mix, blotted dry, and incubated at

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67 to 69°C for 12 hr. Under these conditions, it was found that over 90% of the unlabeled DNA was fixed to the filter pad and that approximately 30% of the labeled homologous DNA was bound to unlabeled DNA, whereas less than 2% of the labeled DNA was bound to filters with unlabeled DNA from organisms belonging to genera other than *Aerococcus*. The amount of labeled DNA bound to unlabeled DNA from the same bacterial strain (a homologous cross) was considered 100% homology for that particular experiment. The relative amount bound to unlabeled DNA from other bacterial strains (heterologous crosses) was considered to be a relative measure of the homology with these strains.

RESULTS

DNA base composition. Each batch of DNA that was purified for these studies was subjected to thermal denaturation, and the DNA base composition was calculated from the absorbancy-temperature profile. The results are summarized in Table 1. All strains that had been classified as *Aerococcus* had 37.0 to 40.2% GC except for strain MK-165 which had only 35.8% GC. Two cultures (*Aerococcus* C₂ and *G. tetragen*a 10875) gave results that differed from values previously reported from this laboratory (5). The two *Gaffkya* cultures were not distinguishable from the *Aerococcus* cultures, although the *G. tetragen*a had a GC content slightly below the aerococci in the present study. The *Pediococcus* strains gave evidence of species differences, with *P. pentosaceus* having a GC content slightly below that of the aerococci and the two strains of *P.*

acidilactici having a GC content slightly above that of the aerococci.

DNA-DNA hybridization. In Table 2 are presented data for four strains of aerococci for which complete reciprocal crosses have been repeated at least twice. The values for the six reciprocal crosses ranged from agreement within 1% (301 versus 303) to 15% (C₆ versus 302). For eight other pairs that have been crossed reciprocally at least twice, there also is agreement within 2 to 15%. Furthermore, in all of the 14 reciprocal crosses, the average per cent homology fell within the range of 69 to 100%.

Table 3 summarizes in a qualitative manner the results of our hybridization experiments. All of the *Aerococcus* cultures tested, except MK-165, generally gave homology values of 69 to 100% when crossed with other aerococci. In 13 crosses between MK-165 and other aero-

TABLE 2. DNA homology of reciprocal hybridizations

Unlabeled, fixed phase (strain)	Labeled, mobile phase (% homology)			
	C ₆	301	302	303
C ₆	100	97	90	92
301	95	100	86	76
302	75	89	100	70
303	86	75	82	100

TABLE 1. Source and DNA base composition of bacterial strains used in this study

Bacterial strain	% GC	Bacterial strain	% GC
<i>Gaffkya tetragen</i> a 10875 ^a	33.0	<i>A. viridans</i> MK-95 ^b	38.5
<i>Pediococcus pentosaceus</i> P1 ^c	35.6	<i>A. viridans</i> 11563 ^{a,d}	38.5
<i>Aerococcus viridans</i> MK-165 ^b	35.8	<i>A. viridans</i> MK-3 ^b	38.7
<i>A. viridans</i> MK-132 ^b	37.0	<i>A. viridans</i> 301 ^e	38.8
<i>A. viridans</i> MK-146 ^b	37.1	<i>A. catalasicus</i> C ₉ ^f	38.9
<i>A. viridans</i> C ₂ ^f	37.1	<i>A. catalasicus</i> C ₆ ^f	39.4
<i>Streptococcus faecalis</i> R-53 ^b	37.5	<i>A. viridans</i> 303 ^e	39.9
<i>A. viridans</i> 302 ^e	37.6	<i>A. viridans</i> C ₇ ^f	40.2
<i>A. viridans</i> MK-53 ^b	37.6	<i>Pediococcus acidilactici</i> SP-7 ^c	42.2
<i>A. viridans</i> MK-201 ^b	37.6	<i>P. acidilactici</i> SP-4 ^c	43.3
<i>Gaffkya homari</i> 10400 ^a	37.9		

^a Culture obtained from American Type Culture Collection, Rockville, Md.

^b Culture maintained in culture collection, Department of Microbiology, North Carolina State University, Raleigh, N.C.

^c Culture obtained from R. Whittenbury, School of Agriculture, Edinburgh, Scotland.

^d Type strain.

^e Culture obtained from A. C. Baird-Parker, Unilever Research Laboratory, Sharnbrook, Bedford, U.K.

^f Culture obtained from O. G. Clausen, University of Oslo, Blindern, Norway.

TABLE 3. Summary of DNA-DNA hybridization data

Cultures	Aerococci	MK-165	<i>Gaffkya homari</i>	<i>Gaffkya tetragena</i>	Pediococci
Aerococci (15) ^a (except MK-165)	69-100 ^b				
Strain MK-165	30-56				
<i>Gaffkya homari</i>	40-95	41-55			
<i>Gaffkya tetragena</i>	2-9	3	6-9		
Pediococci (3) ^a	3-12	1	1	8	
<i>Streptococcus faecalis</i>	2-6	2-5	3	2-3	2-3

^a Number of strains tested.^b Range of values, including reciprocal crosses. All figures shown represent percentages.

cocci, the homology values range from 30 to 56%. The culture of *G. homari* in 24 crosses with aerococci gave values of 40 to 95%, with an average of 74% homology. However, the *G. tetragena* showed only 2 to 9% homology with aerococci and only 6 to 9% with the *G. homari* strain. One culture of *Streptococcus faecalis* in 14 crosses with aerococci indicated only 2 to 6% homology, and three cultures of *Pediococcus* in 10 crosses with aerococci gave 3 to 12% homology.

DISCUSSION

Both the DNA base composition data and the hybridization studies support the concept of *A. viridans* as a biologically valid species. The one strain of *G. homari* that was included in this study appeared to belong in the same species as *A. viridans*, as suggested on the basis of physiological studies (2). Like many strains that have been maintained in culture collections over a period of years, this organism grows more slowly and is less active in a variety of physiological tests when compared to organisms that have been isolated more recently.

Strain MK-165 has a DNA base composition slightly below that of typical *A. viridans* and its DNA hybridizes somewhat less readily. Nutritional studies (8) also suggest minor differences between this strain and typical *A. viridans*. For the present it seems advisable to withhold judgment as to whether this culture may belong to a second species of *Aerococcus* or is simply an odd strain of *A. viridans*.

There is no evidence of close DNA homology between the aerococci and the strains of pediococci, the enterococcus, or the *G. tetragena* that were included in this study.

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