Deoxyribonucleic Acid Homology among Strains of the Lobster Pathogen '*Gaffkya homari*' and *Aerococcus viridans*

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(Received 3 July 1973; revised 1 November 1973)

Gaffkya homari, a Gram-positive tetracoccus that causes a fatal septicaemia in lobsters, was first described by Hitchner & Snieszko (1947). Subsequently Wood (1965) reported the isolation of both pathogenic and non-pathogenic strains of *G. homari* from lobsters; the pathogenicity of this species for lobsters has been confirmed (Rabin, 1965; Stewart, Foley & Ackman, 1969; Puzniak, Chesbro & Evans, 1971; Tubiash & Krantz, 1970).

There has been considerably less agreement concerning the phenotypic characteristics and taxonomic relationships of this organism. However, most such studies have been relatively cursory and largely limited to only one or two strains (Aaronson, 1956; Deibel & Niven, 1960; Evans & Schultes, 1969; Miller & Evans, 1970; Stewart *et al.* 1969; Wood, 1965). The detailed procedures and results in most instances were not described. The most comprehensive study was that of Deibel & Niven (1960), who included two strains of *Gaffkya homari* in their study of Gram-positive tetracocci. Even though these two cultures differed from each other in 12 of the 39 tests reported, it was concluded that they were indistinguishable from *Aerococcus viridans* and that the correct name for the species should be *Pediococcus homari*. Other authors have generally concurred in the inclusion of *G. homari* and *A. viridans* in a single species even though they disagree concerning the appropriate genus (Schultes & Evans, 1971). The choice has been narrowed by the recent official rejection of the genus name *Gaffkya* (Editorial Secretary, 1971). A close relationship to the genus *Pediococcus* is not supported by DNA homology studies (Schultes & Evans, 1971), vitamin requirements (J. B. Evans, unpublished), or peptidoglycan composition (Nakel, Ghuysen & Kandler, 1971). There seems to be no valid basis for considering this species to be any closer to the genus *Pediococcus* than it is to some other genera of Gram-positive cocci, and we believe it should be retained in the separate genus *Aerococcus*. The present study was designed to determine by means of DNA hybridization the homology among the lobster and non-marine strains of *A. viridans*.

METHODS

Cultures and growth conditions. The bacterial strains used in this study and their sources are included in Table 1. Cultures for DNA extraction were grown in Trypticase Soy Broth (BBL, Division of Becton, Dickinson and Co.) at 30 °C for 17 to 24 h, depending on the growth rates of the different cultures. Labelled DNA was produced by incorporation in the growth medium of 0.33 µCi/ml methyl-[³H]thymidine (New England Nuclear).

DNA base composition. For each culture unlabelled DNA was extracted and purified as described previously (Schultes & Evans, 1971). The amount of DNA was determined by measuring extinction at 260 nm using a standard curve based on salmon sperm DNA.

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The moles percentage of guanine plus cytosine was calculated from the thermal denaturation curve with the aid of a computer program developed as part of this study and based upon the method of Marmur & Doty (1962).

DNA-DNA hybridization. Unlabelled DNA was denatured by heating for 10 min at 100 °C in SSC (0.15 M-NaCl and 0.015 M-sodium citrate, pH 7.0) and 100 µg of this DNA in 0.4 ml of SSC was added to nitrocellulose membrane filters (type B-6, Schleicher and Schuell Co.) that had been soaked overnight in sixfold concentrated SSC at 4 °C. The filters were washed with sixfold concentrated SSC, dried, cut in quarters, and vacuum-dried in scintillation vials. One ml of Denhardt's (1966) preincubation mixture with 30 % dimethylsulphoxide (Rogul, Brendle, Haapala & Alexander, 1970) was added to each vial and incubated at 60 °C for 4.5 h. The filter quarters were blotted dry and returned to the vials from which the preincubation mixture had been poured. Labelled DNA from the reference strains was denatured and sheared as described previously (Schultes & Evans, 1971), and 0.5 µg samples in 0.5 ml of twofold concentrated SSC added to appropriate filter quarters. These were incubated at 60 °C for 16 h, placed in an ice bath, washed with twofold concentrated SSC+30 % (w/v) dimethylsulphoxide, and washed with twofold concentrated SSC at 60 °C. After drying, the amount of labelled DNA bound on each filter was determined in a scintillation counter. Controls for non-specific binding and a homologous cross were included in each experiment. The percentage homology for heterologous crosses was calculated by considering the homologous cross equal to 100 % homology.

RESULTS AND DISCUSSION

In DNA base composition the six *Gaffka homari* strains appear to have a slightly higher percentage GC than the three *Aerococcus viridans* strains (Table 1). However, other studies in our laboratory have shown that other strains of *A. viridans* give values that completely overlap those for *G. homari*.

In DNA-DNA hybridization experiments two reference strains of *Gaffkya homari* and one of *Aerococcus viridans* were used as sources of the labelled, sheared DNA in the mobile phase (Table 1). The very high degree of homology among the six strains from lobsters is shown with all three reference strains. With the two *G. homari* reference strains there was 93 to 97 % homology with the other lobster strains and all six strains had 53 to 57 % homology with the *A. viridans* reference strain.

The results also show a close relationship between the two groups of organisms. The strains of *Aerococcus viridans* had 76 to 86 % homology with the two reference strains of *Gaffkya homari* – which was actually closer than their relationship to the reference strain of *A. viridans* (71 to 73 %). However, this homology among the *A. viridans* strains was closer than that between the *A. viridans* reference strain and the six strains of *G. homari* (53 to 57 %).

The low homology values found for the three control organisms with all three reference strains serve to emphasize the relatively close relationship between the two groups of tetracocci. The *Streptococcus faecalis* strain had a DNA base composition that was within the range of values for the tetracocci and the *Staphylococcus epidermidis* strain had only a slightly lower percentage of guanine plus cytosine in its DNA. Yet under the conditions of the present study these other Gram-positive cocci, that also share numerous phenotypic similarities with the tetracocci, had homology values in the same range as did *Escherichia coli* and as reported previously for three strains of pediococci (Schultes & Evans, 1971).

The high degree of homology among the lobster strains in contrast to the greater variation among the non-marine aerococci may well be a reflection of more constant environmental

Table 1. *DNA homologies between strains of Gaffkya homari and Aerococcus viridans*

Cultures used as source of unlabelled DNA	Percentage G + C	Percentage bound DNA of reference strain		
		wc9	6870	MK201
<i>G. homari</i> wc7*	38.6	94 ± 4	97 ± 2	57 ± 5
<i>G. homari</i> wc9*	38.4	100 ± 0	97 ± 6	57 ± 2
<i>G. homari</i> wc10*	38.8	95 ± 4	97 ± 7	55 ± 5
<i>G. homari</i> 6870*	37.5	96 ± 5	100 ± 0	53 ± 1
<i>G. homari</i> HT1†	38.2	96 ± 1	93 ± 3	56 ± 2
<i>G. homari</i> 10400‡	37.7	95 ± 1	95 ± 6	53 ± 2
<i>A. viridans</i> MK201§	37.0	80 ± 6	81 ± 5	100 ± 0
<i>A. viridans</i> 303	36.4	86 ± 1	78 ± 1	73 ± 3
<i>A. viridans</i> LS17¶	35.5	79 ± 7	76 ± 8	71 ± 3
<i>S. epidermidis</i> BP31	32.5	4 ± 2	8 ± 1	7 ± 2
<i>S. faecalis</i> H27a**	36.9	2 ± 1	5 ± 1	6 ± 3
<i>E. coli</i> K12‡	48.9	2 ± 1	4 ± 1	4 ± 2

Relations among strains are expressed as percentages relative to the homologous reaction which is taken as 100 %. Three independent determinations were conducted with DNA from each reference strain and the range of reproducibility is shown.

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‡ American Type Culture Collection, Rockville, Maryland, U.S.A.

§ Isolated in this laboratory by M. A. Kerbaugh from a hospital environment.

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parameters in the normal habitat of the lobster strains. Certainly there is strong evidence that both groups belong to the same species and, if one wishes to separate them, the lobster strains might be considered as a subspecies of the broader species (i.e. *Aerococcus viridans* subsp. *homarus*). Support for such a separation comes from the report of Puzniak *et al.* (1971) that strains of *A. viridans* from hospital sources failed to exhibit pathogenicity when injected into lobsters, as well as from the DNA homology studies presented here.

This investigation was supported in part by Public Health Service Grant AI-07693 and Training Grant GM-01265 from the National Institute of General Medical Sciences. We thank all those who contributed cultures, and Constance Puzniak and W. R. Chesbro for data on physiological and virulence tests.

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