Deoxyribonucleic Acid Reassociation Between *Actinomyces denticolens* and Other *Actinomyces* Species from Dental Plaque

V. E. DENT† AND R. A. D. WILLIAMS*

Department of Biochemistry, The London Hospital Medical College, London, E1 2AD, United Kingdom

The status of the new species *Actinomyces denticolens* was established by deoxyribonucleic acid-deoxyribonucleic acid homology. The level of homology with *Actinomyces naeslundii*, *Actinomyces viscosus*, and *Actinomyces howellii* was low in each case.

Actinomyces denticolens has been proposed as a new taxon for strains which have been isolated from the dental plaque of cattle and which have physiological similarities to *Actinomyces naeslundii* but are different in electrophoretic polypeptide pattern and cell wall carbohydrate components. Deoxyribonucleic acid (DNA)-DNA homology confirms the distinction among *A. denticolens*, *A. naeslundii*, and *Actinomyces howellii*.

Among the predominant components of the bacterial flora of dental plaque from a wide range of mammals, *Actinomyces* isolates were found in virtually all of the individuals (3). The strains isolated were all presumptively identified as either *Actinomyces viscosus* or *A. naeslundii* on the basis of physiological properties (9). Among the putative strains of *A. naeslundii*, 35 isolates had sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns similar to those of culture collection strains of this species (Dent and Williams, unpublished data), but the isolates from dairy cattle had several distinct patterns of polypeptides. Strains which had only rhamnose as a cell wall carbohydrate, failed to ferment trehalose, and fermented mannose weakly were described as *A. denticolens* (4), and isolates which contained glucose and rhamnose in their cell walls and were less widely fermentative than *A. naeslundii* were allocated to another new species, *A. howellii* (5). In this paper we report the results of DNA-DNA homology studies with *A. denticolens* and representatives of other *Actinomyces* species with similar DNA mean base compositions and physiological properties.

The strains used and their sources are listed in Table 1. DNA was isolated and purified as described previously for *A. howellii* (5). The mean base composition was determined from the melting temperature in dilute saline citrate by using the following formula: guanine-plus-cytosine content = $50.9 + 2.08(\text{melting temperature of unknown} - \text{melting temperature of } Escherichia \text{ coli strain B DNA})$ (7). DNA type VIII (catalog no. D2001; Sigma London Ltd.) was used as an internal standard. The guanine-plus-cytosine content of the DNA (65.9 to 67.7 mol%) was similar to the guanine-plus-cytosine contents of DNAs of *A. naeslundii* (9), *A. viscosus* (2), and *A. howellii* (5), but distinct from the guanine-plus-cytosine contents of DNAs of *Actinomyces bovis*, *Actinomyces israelii*, and *Actinomyces odontolyticus* (9).

For homology studies DNA was purified by cesium chloride density gradient centrifugation and labeled by nick translation with ^3H -labeled deoxycytidine triphosphate (kit TRK 700; Amersham International), and homology determinations were carried out on 5-mm nitrocellulose membrane disks (1, 10). Our results (Table 2) show that the strains described as *A. denticolens* (4) form a homology group which has low DNA-DNA homology with recent isolates of *A. naeslundii* from the dental plaque of primates (strains BB1/1958 and SM/1643) and with strain WVU 45, which is equivalent to the type strain of this species. Similar low levels of homology were observed between *A. denticolens* and the type strain of *A. howellii* and between *A. denticolens* and strain T6, which is equivalent to the type strain of *A.*

TABLE 1. Designations and sources of *Actinomyces* strains

Strain	Origin	Source
<i>A. denticolens</i> NCTC 11490 ^T (= Sh8/4303 ^T) ^a Sh8/4313 Sh5/4040 Sh7/4277 Sh7/4250	Dental plaque of dairy cattle	V. E. Dent, London Zoo
<i>A. naeslundii</i> BB1/1958 SM/1643 WVU 45 ^T (= ATCC 12104 ^T)	Dental plaque of bush baby Dental plaque of spider monkey Sinus after dental extraction	V. E. Dent, London Zoo V. E. Dent, London Zoo G. H. Bowden, Winnipeg, Canada
<i>A. viscosus</i> T6 ^T (= ATCC 15987 ^T)	Hamster mouth	G. H. Bowden, Winnipeg, Canada
<i>A. howellii</i> NCTC 11636 ^T (= Sh7/4276 ^T)	Dental plaque of dairy cattle	V. E. Dent, London Zoo

^a T = type strain.

* Corresponding author.

† Present address: Department of Botany, Queens University, Belfast, BT7 1NN, United Kingdom.

viscosus. It is known that *A. viscosus* is heterogeneous serologically (6) and in DNA-DNA homology (2), although it has much in common with *A. naeslundii* physiologically (8). Despite this, it was not necessary to conduct hybridizations

TABLE 2. DNA relatedness values among *A. denticolens* and other *Actinomyces* species

Strain	Guanine-plus-cytosine content of DNA (mol%)	% Relatedness to strain:						
		Sh5/4040	Sh7/4277	BB1/1958	SM/1643	WVU 45 ^T	T6 ^T	Sh7/4276 ^T
<i>A. denticolens</i>								
NCTC 11490 ^T (= Sh8/4303 ^T)	66.7	103	77	19	33	39	21	30
Sh8/4313	66.7	88	73	19	27	29	21	25
Sh5/4040	67.7	100	88	20	27	36	25	28
Sh7/4277	67.5	89	100	25	26	32	33	24
Sh7/4250	65.9	99	97	21	32	38	26	32
<i>A. naeslundii</i>								
BB1/1958	65.9	20	21	100	NT ^a	84	46	NT
SM/1643	64.8	29	NT	NT	100	99	NT	24
WVU 45 ^T (= ATCC 12104 ^T)	64.0	26	NT	NT	NT	100	NT	NT
<i>A. viscosus</i>								
T6 ^T (= ATCC 15987 ^T)	66.1	28	26	46	NT	67	100	29
<i>A. howellii</i>								
NCTC 11636 ^T (= Sh7/4276 ^T)	66.7	34	NT	NT	31	20	20	100
<i>Lactobacillus odontolyticus</i>								
NCTC 1407		3	1	4	2	1	3	2

^a NT, Not tested.

with strains other than type strains, because *A. denticolens* is clearly distinguishable from both serotypes of *A. viscosus* and from both "typical" and "atypical" strains of *A. naeslundii* (6) by its unique and simple cell wall carbohydrate content and also its strikingly different electrophoretic pattern of polypeptides (4). Therefore, our data confirm the status of *A. denticolens* as a distinct new species.

This work was supported by project grants G979295SB and G8207598SB from the Medical Research Council.

LITERATURE CITED

1. Back, W., and E. Stackebrandt. 1978. DNS-DNS-Homologie-Studien innerhalb der Gattung *Pediococcus*. Arch. Microbiol. 118:79-85.
2. Coykendall, A. L., and A. J. Munzenmaier. 1979. Deoxyribonucleic acid hybridization among strains of *Actinomyces viscosus* and *Actinomyces naeslundii*. Int. J. Syst. Bacteriol. 29:234-240.
3. Dent, V. E., and P. D. Marsh. 1981. Evidence for a basic plaque microbial community on the tooth surface in animals. Arch. Oral Biol. 26:171-179.
4. Dent, V. E., and R. A. D. Williams. 1984. *Actinomyces denticolens* Dent & Williams sp. nov.: a new species from the dental plaque of cattle. J. Appl. Bacteriol. 56:183-192.
5. Dent, V. E., and R. A. D. Williams. 1984. *Actinomyces howellii*, a new species from the dental plaque of dairy cattle. Int. J. Syst. Bacteriol. 34:316-320.
6. Fillery, E. D., G. H. Bowden, and J. M. Hardie. 1978. A comparison of strains of bacteria designated *Actinomyces viscosus* and *Actinomyces naeslundii*. Caries Res. 12:299-312.
7. Owen, R. J., and L. R. Hill. 1979. The estimation of base compositions, base pairing and genome sizes of bacterial deoxyribonucleic acids. Soc. Appl. Bacteriol. Tech. Ser. 14:277-296.
8. Schofield, G. M., and K. P. Schaal. 1981. A numerical taxonomic study of members of the *Actinomycetaceae* and related taxa. J. Gen. Microbiol. 127:237-259.
9. Slack, J. M., and M. A. Gerencser. 1975. *Actinomyces*, filamentous bacteria. Biology and pathogenicity. Burgess Publishing Co., Minneapolis.
10. Stackebrandt, E., and F. Fiedler. 1979. DNA-DNA homology studies among strains of *Arthrobacter* and *Brevibacterium*. Arch. Microbiol. 120:289-295.