Strain Differentiation of *Rothia dentocariosa* and Related Isolates by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

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Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis was used to compare soluble protein fractions from 36 strains of *Rothia dentocariosa* and related bacteria. The resulting protein patterns were examined to identify the bands of major and minor frequency within the genus. A cluster analysis revealed three major *Rothia* groups and a fourth group representing the genus *Actinomyces*. Our data demonstrated good correlations (>70%) with existing biotyping and serotyping schemes of these isolates. We suggest that electrophoretic group 2 represents a new *Rothia* species.

The genus *Rothia* was created by Georg and Brown (4) to accommodate aerobic, catalase-positive actinomycetes which did not fit into either *Actinomyces* or *Nocardia*. This genus contains one species, *Rothia dentocariosa*. Since its conception, numerous strains have been isolated from oropharynges, dental plaque, and, occasionally, infectious processes (2, 8, 11, 16). A number of isolates of *Rothia*-like bacteria which are biochemically or serologically different than *R. dentocariosa* have been encountered. The taxonomic status of these *Rothia*-like organisms is uncertain.

A number of investigators (3, 6, 12, 13) have shown that polyacrylamide gel electrophoresis (PAGE) of soluble proteins can be used to differentiate species of bacteria. Taxonomic groupings based on PAGE of proteins agree closely with groupings based on deoxyribonucleic acid-deoxyribonucleic acid homology (1, 10, 14). Therefore, PAGE of soluble proteins can be a useful and reliable taxonomic tool.

In this study, sodium dodecyl sulfate-PAGE was used in an attempt to resolve some of the taxonomic uncertainty associated with strains of *R. dentocariosa* and *Rothia*-like organisms. In addition, several representatives of the genus *Actinomyces* were examined. The electrophoretic patterns obtained were used to measure strain similarity among the *Rothia* isolates and to demonstrate differences between *Rothia* and *Actinomyces* species.

MATERIALS AND METHODS

Organisms and growth conditions. The *Rothia* and *Rothia*-like strains used in this study are listed in Table 1. Stock cultures were maintained on Trypticase-soy agar (BBL Microbiology Systems, Cockeysville, Md.) slants and were transferred monthly. For electrophoresis, cultures were grown aerobically at 35°C in 1 liter of Trypticase-soy broth (BBL) in 2-liter flasks on a shaker; these cultures were harvested at mid-log phase (determined spectrophotometrically) by centrifugation.

Sample preparation. After harvesting, the cells from each culture were washed twice in chilled tris-(hydroxymethyl)aminomethane buffer (Sigma Chemical Co., St. Louis, Mo.), pH 8.9. Approximately 30.0 ml of washed, packed cells was combined with 20.0 ml of glass beads (diameter, 0.1 mm) and disrupted with a Braun homogenizer (Glasperlén; Melsungen AG, West Germany) maintained at 4°C. The resulting homogenates were then centrifuged at 3,000 × *g* to remove the beads and any remaining whole cells. The supernatants were

centrifuged at 38,000 × *g* for 1 h to yield the soluble fractions.

The supernatants from the high-speed centrifugation were

TABLE 1. Strains used in this study

Organism	WVU strain no. ^a	Other designation and/or source ^a
<i>R. dentocariosa</i>	477	CDC W808, leg stump drainage
	478	CDC W876, blood
	858	WVU isolate, dental plaque
	1200	WVU isolate, dental plaque
	1489 ^T	ATCC 17931 ^T (neotype strain)
	1524	ATCC 14189, carious teeth
	1525	ATCC 14190, carious teeth
	1526	ATCC 14191, carious teeth
	1534	CDC W1581
	1549	CDC W1578, sputum
	1555	CDC X359
	1603	CDC X347
	1604 ^T	ATCC 17931 ^T (neotype strain)
	1605	ATCC 19426, type strain of <i>Nocardia salivae</i> ^b
	1610	CDC W3324, urine
	1611	CDC D7154, abdominal wound
	1612	CDC E4554, salivary gland drainage
	1613	CDC W585
<i>Rothia</i> -like strains	841	WVU isolate, dental plaque
	918	WVU isolate, dental plaque
	936	WVU isolate, dental plaque
	972	WVU isolate, dental plaque
	997	WVU isolate, dental plaque
	1073	WVU isolate, dental plaque
	1556	CDC X483, blood
<i>Actinomyces israelii</i>	1557	CDC W781, throat
	46 ^T	ATCC 12102 ^T (neotype strain)
<i>Actinomyces naeslundii</i>	45	ATCC 12104, reference strain
	777	S. Socransky strain I
<i>Actinomyces viscosus</i>	820	S. Bellack N16, dental plaque
	371	ATCC 19246, human actinomycetes
	626	C. S. Cumins, VPI 3426
<i>Actinomyces odontolyticus</i>	745 ^T	ATCC 15987 ^T
	482	WVU isolate, dental plaque
<i>Actinomyces bovis</i>	750	CDC W1043
	116 ^T	ATCC 13683 ^T (neotype strain)

^a WVU, West Virginia University Culture Collection, Morgantown; CDC, Centers for Disease Control, Atlanta, Ga.; ATCC, American Type Culture Collection, Rockville, Md.; VPI, Virginia Polytechnic Institute and State University, Blacksburg.

^b Originally described as the type strain of *Nocardia salivae*. *N. salivae* is a synonym of *R. dentocariosa*.

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Constant application of 150 V (model 500/200 power supply; Bio-Rad) for 4 h resulted in a dye front migration of about 7.5 cm. The chamber buffer was maintained at a temperature of 10°C.

The gels were stained for 2 h in 50% methanol–7% acetic acid–1% (wt/vol) Coomassie blue R-250 (Bio-Rad); this was followed by a 24-h destaining period in 5% glacial acetic acid with stirring in a diffusion destainer (model 22; Bio-Rad).

Data analysis. The resulting slab gels were photographed, and the protein band migration distances were measured and compared from the photographs. The band migration distances were divided by the migration distance of bovine serum albumin, which yielded relative distance values. This served to normalize banding patterns, allowing inter- and intrarun comparisons. A composite of all 36 strains was compiled on a cue sheet for conversion to a binary code, with 1 denoting presence and 0 denoting absence of a band

FIG. 2. Phenogram of relationships among various *Rothia* and *Actinomyces* strains clustered by unweighted pair group averaging. The scale represents similarity (simple matching) coefficients.

for computer programming. The similarity coefficient was based on simple matching and incorporated a rectangular 36 × 64 matrix with unweighted pair group analysis, using arithmetic averages (5). This resulted in the clustering of the 36 strains in decreasing order of similarity with respect to the 64 possible band positions for each strain.

RESULTS AND DISCUSSION

The normalized electrophoretic patterns obtained for *Rothia* and *Actinomyces* proteins are shown in Figure 1.

The *Rothia* strains demonstrated a common genus band at position 39, with one exception (strain WVU 1200). Bands at positions 26, 32, 48, and 53 occurred in at least 20 of the 26 *Rothia* strains tested. Other bands that appeared in 16 of the 26 *Rothia* strains included the bands at positions 12, 22, 43, 52, 58, and 61.

Although no common band was observed in the 10 strains of *Actinomyces* examined, band 36 was found in all strains except the *Actinomyces bovis* strain, and band 54 appeared in all strains except *Actinomyces viscosus* serotype 1 (strain WVU 745^T). Other bands evident in 8 of the 10 strains included the bands at positions 2, 4, 16, 35, 40, and 45. In 7 of the 10 *Actinomyces* strains studied, bands were observed at positions 19, 24, 25, 43, 48, and 53.

A cluster analysis of the protein band patterns identified

four major groups (Fig. 2). Cluster 4 contained all *Actinomyces* strains, which were clearly separable from the *Rothia* strains (clusters 1, 2, and 3) at a similarity level of 52%. Since the number of strains examined was limited, no additional conclusions about relationships within the genus *Actinomyces* were reached. However, it should be noted that one strain of *A. viscosus* serotype 1 (strain WVU 745^T) did show some relationship to two strains of serotype 2 (strains WVU 371 and WVU 626); similarity coefficients, 65 and 67%, respectively). *A. viscosus* WVU 371 and WVU 626 were more closely related (similarity level, 76%).

The largest *Rothia* group, cluster 1, contained 11 strains. The levels of similarity within this cluster ranged from 76 to 95%. Strains WVU 1489 and WVU 1604 are duplicate representatives of the type strain of *R. dentocariosa*, strain ATCC 17931. These strains showed a similarity coefficient of 92%. Because the type strain is a member of cluster 1, we believe that this cluster represents the species *R. dentocariosa*.

Leshner et al. (7) divided *Rothia* into four biotypes and three serotypes. Figure 3 shows the relationship of these biotypes and serotypes to the PAGE data for those strains which demonstrated similarity levels of 75% or more. All cluster 1 strains belong to biotype 1 but fall into serotypes 1 and 2. This suggests that *R. dentocariosa* is homogeneous biochemically but has more than one serotype. On the other hand, a comparison of these data with the numerical taxonomic data of Schofield and Schaal (15) indicates that there is also biochemical variation in *R. dentocariosa*. Schofield and Schaal identified two subclusters in the genus *Rothia* on the basis of morphological and physiological characters. Two strains belonging to subcluster 16a and one strain belonging to subcluster 16b were used in our study. All three strains belonged to sodium dodecyl sulfate-PAGE cluster 1.

The second *Rothia* cluster, cluster 2, contained nine strains; the intracluster levels of similarity ranged from 75 to 90%. Cluster 2 was related to cluster 1 at a level of 63%. Cluster 2 strains belong primarily to biotypes 3 and 4, which are biochemically different from typical *R. dentocariosa*. Serologically, five of the strains belong to serotype 3, three strains belong to serotype 1, and one strain remains untyped. Further study of the strains that are now placed in serotype 1 may show that they are actually different from serotype 1 strains. This has been done for strain WVU 1603, which was originally identified as serotype 1. Reciprocal absorption studies have indicated that this strain has a strong cross-reaction with, but is different from, serotype 1 strains (unpublished data). On the basis of biochemical and serological reactions, strain WVU 1603 was thought to resemble strain WVU 1604. The results of the PAGE studies showed that these strains are different.

Since taxonomic groupings based on electrophoresis of proteins closely resemble groupings based on deoxyribonucleic acid-deoxyribonucleic acid homology studies, we suggest that PAGE cluster 2 represents a second species of the genus *Rothia*.

The third and smallest *Rothia* cluster contained only five strains. Cluster 3 had lower levels of intracluster similarity than the other *Rothia* clusters. The levels of similarity within cluster 3 ranged from 63 to 82%. Cluster 3 strains were only 58% related to cluster 1 and 2 strains. Cluster 3 strains also exhibited heterogeneity in biochemical and serological reactions. Serologically, one strain, strain WVU 1200, was identified as serotype 2, and one additional strain cross-reacted with both serotype 1 and serotype 2 strains. The remaining three strains did not react with the available

FIG. 3. Biotype and serotype matches in *Rothia* strains with PAGE similarity coefficients of 75% or greater. (Biotypes and serotypes were determined by Leshner et al. [7].)

antisera. This cluster is too small and diverse to make any suggestions concerning its species status at the present time.

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