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Characterization of *Wolinella* spp., *Campylobacter concisus*, *Bacteroides gracilis*, and *Eikenella corrodens* by Polyacrylamide Gel Electrophoresis

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The small asaccharolytic, nonpigmenting gram-negative rods of the human oral cavity are difficult to differentiate from each other. Protein profiles of sonicated cells of *Wolinella* species, *Campylobacter concisus*, *Bacteroides gracilis*, and *Eikenella corrodens* were obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and visualized with a silver stain. The gels were scanned with a laser densitometer, and the similarity of strains was computed by determining correlation coefficients of normalized densities along the gels. The strains were grouped by cluster analysis of the correlation coefficients. All species were distinct from each other. Several groups were found within *E. corrodens*. A colored silver stain was found to highlight species differences and appears to be useful in the rapid identification of fresh isolates.

Data from various laboratories have indicated relationships between *Wolinella recta*, *Bacteroides gracilis*, and *Eikenella corrodens* and progressive periodontal (active) disease (2, 11; A. C. R. Tanner, J. L. Dzink, J. L. Ebersole, and S. S. Socransky, submitted for publication). *B. gracilis* has been described as a pathogen in clinical infections (3).

W. recta, *Wolinella curva*, *Campylobacter concisus*, *B. gracilis*, and *E. corrodens* are distinct from each other genetically and serologically (1, 8, 9; A. C. R. Tanner and J. L. Ebersole, Dent. Res. **61**:232, abstr. no. 482, 1982). However, differentiation and identification of these species, especially *C. concisus*, *W. curva*, and *B. gracilis* can be difficult because they have a similar metabolism and are resistant to dyes and inhibitors (8).

In this investigation, protein profiles obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole cell proteins of *Wolinella* species, *B. gracilis*, *C. concisus*, and *E. corrodens* were compared. If the species could be adequately differentiated by SDS-PAGE, this would lead to a more reliable and rapid method for species identification.

MATERIALS AND METHODS

The strains examined were isolated mainly from diseased periodontal sites (Table 1) and were included in the taxonomic studies describing the species (8, 9). The 28 isolates for intensive study were selected from 61 reference strains. The isolates included representatives of all protein profile types within each species that were observed in preliminary electrophoretic runs. The strains were maintained on Trypticase soy agar plates supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) and were incubated anaerobically.

Preparation of strains for SDS-PAGE, SDS-polyacrylamide gel preparation, and running of the samples were performed as previously described (10). Cells were scraped from the surfaces of Trypticase soy agar plates (supplemented with 5% sheep blood), sonicated for 1 min, and boiled for 5 min. Cell preparations were loaded onto an 11% SDS-polyacrylamide gel with a stacking gel containing 4% acrylamide. Electrophoresis was carried out at 15 mA/0.75-mm gel and typically took 3 to 5 h.

For densitometric analysis, trypsin inhibitor (molecular size, 20,000 daltons [Da]) was included as an internal standard with each bacterial preparation. Proteins were visualized with a silver stain (conventional silver stain) (10) and scanned with a laser densitometer linked to a computer (4, 10). Bacterial protein profiles were compared by using cluster analysis (8) of the correlation coefficients of peak densities measured by the densitometer.

Cell preparations were also run on SDS-polyacrylamide gels without internal standards and stained with a colored silver stain (6, 7) (Gelcode colored silver stain; Pierce Chemical Co., Rockford, Ill.). This process involved complexing silver with polypeptide-reactive centers. The reaction was initiated by placing a polypeptide-containing gel, previously equilibrated with an appropriate concentration of silver nitrate, into a reducing solution that contained sodium hydroxide, sodium borohydride, and formaldehyde. After an appropriate time in the reducing solution, the gel was equilibrated through two changes of an enhancing solution that contained sodium carbonate (6, 7). This gel was examined for distinctive colored bands, which could be used in the identification of fresh isolates.

RESULTS

Conventional silver stain. The protein profiles of the strains within each of the formate- and fumarate-utilizing species *W. recta*, *W. curva*, *C. concisus*, and *B. gracilis* were different from each other (Fig. 1). These species showed greater than 73% intraspecies similarity by cluster analysis of correlation coefficients (Fig. 2) when run on the same gel (Table 2) or when run on two different gels (Table 3). Intraspecies similarities were consistently at a lower level than interspecies similarities, regardless of whether isolates were run on the same or different gels (Tables 2 and 3).

There was heterogeneity of the protein profiles with *E. corrodens* (Fig. 1). Four protein profile patterns within the species were found by cluster analysis (Fig. 2), although the protein profiles were more similar to each other than to those of any other of the species examined (Tables 2 and 3). With this conventional silver stain, *W. recta* could be differentiated by yellow bands midway between the 20,000- and 29,000-Da standards (Fig. 1).

TABLE 1. Sources of strains

Species and strain[a]	Source
Wolinella recta	
ATCC 33238^T (371), 303, and 1219	Periodontal pocket
VPI 10280	Dental root canal
W. curva	
ATCC 35244^T (VPI 9584)	Alveolar abscess
640 and VPI 10659	Human clinical
W. succinogenes	
ATCC 29543 (602W)	Bovine rumen
Campylobacter concisus	
ATCC 33237^T (484), 483, and 522	Periodontal pocket
Bacteroides gracilis	
ATCC 33236^T (1084), 402, and 1083	Periodontal pocket
B. urealyticus	
VPI 7814	Anal fistula
VPI 7815	Human clinical
Eikenella corrodens	
373, 384, 468, 469, 525, 557, 558, 1006, 1007, 1010, 1011, 1073, 1074, and 1079	Periodontal pocket
ATCC 23834	Human clinical

[a] Strains were from the Forsyth Dental Center unless otherwise indicated.

Colored silver stain. The colored silver stain (Gelcode) stained bands of different molecular weights within each species in bright yellow (Fig. 3). *B. gracilis*, *W. curva*, and *C. concisus* showed differently colored highlighted bands between 29,000 and 45,000 Da on gels stained with the colored stain. *B. gracilis* was characterized by a yellow band around 45,000 Da and an orange band below 20,000 Da. *C. concisus* showed five equidistant yellow bands between 29,000 and 45,000 Da, whereas *W. curva* showed four yellow bands in the same region, with one band level with the 45,000-Da standard. Because the protein profiles of the two *Wolinella* species were quite similar in this region, the lower-molecular-weight yellow bands of *W. recta* observed on the conventional silver-stained gels appeared to be a useful differentiating characteristic.

All *E. corrodens* strains showed a bright yellow band below 29,000 Da on gels stained with the colored silver stain. The differentiating bands within this species were yellow and red and were located in the 29,000- to 45,000-Da region.

DISCUSSION

The differentiation of *W. curva*, *C. concisus*, and *B. gracilis* previously relied on a few phenotypic characteristics (motility, growth enhancement in a microaerophilic atmosphere, and serologic reactions). The differentiation of these species from each other by using protein profiles can provide a reliable additional method to aid in the identification of fresh isolates.

My findings extend the observations of Moore et al. (5) that oral species can be grouped by PAGE. With the increased sensitivity of the described methods, one should be able to group isolates and assign identifications. The demonstrated reproducibility of protein banding on two separate gels suggests that identification could be made by cluster analysis or probabilistic identification of scanned gels as well as by observation of species-distinctive colored bands. I recently used protein profiles to identify over 50 *W. recta* strains and 70 *B. gracilis* strains isolated from oral sites. I was able to regroup an atypical *C. concisus* isolate to *W. curva* by using the protein profiles. This method has the additional advantage that the need for broth culturing of these fastidious species is avoided, since cells can be harvested directly from agar plates.

Different protein profile groups were found within *E. corrodens* (Fig. 1 and 2). SDS-PAGE provides a rapid, sensitive method for subgrouping this species, and its use

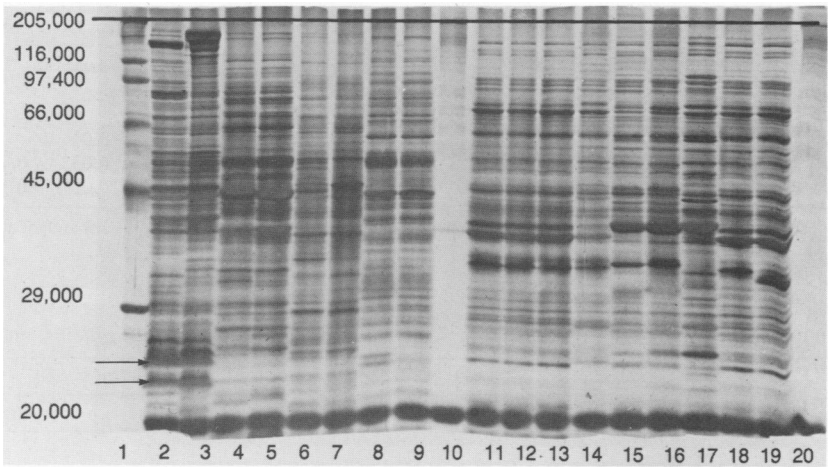

FIG. 1. SDS-PAGE with conventional silver stain of sonicated whole cells of *W. recta*, *W. curva*, *C. concisus*, *B. gracilis*, and *E. corrodens*. All species had different protein profiles. Four different patterns of protein profiles were distinguished for *E. corrodens* strains. The line at 205,000 Da indicates the position of the superimposed high-molecular-weight internal standard. The band in lanes 2 to 20 just below 20,000 Da is the low-molecular-weight trypsin inhibitor internal standard. Arrows indicate the positions of yellow-staining bands of *W. recta* (conventional silver stain). Lanes: 1, high-molecular-weight standard mixture; 2 and 3, *W. recta* ATCC 33238^T and 303, respectively; 4 and 5, *W. curva* ATCC 35244^T and 640, respectively; 6 and 7, *C. concisus* ATCC 33237^T and 522, respectively; 8 and 9, *B. gracilis* ATCC 33236^T and 402, respectively; 10 and 20, myosin and trypsin inhibitor standards, respectively; 11 through 19, *E. corrodens* 1073, 373, 1007, 1011, 468, 469, 558, 384, and 1074, respectively.

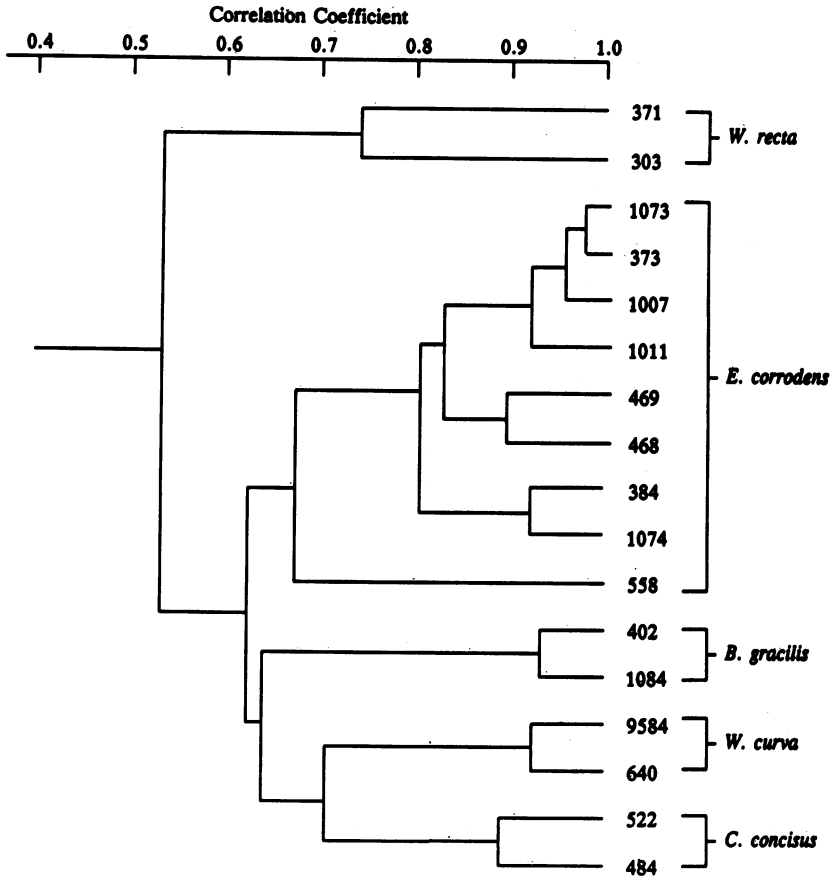

FIG. 2. Cluster analysis of the correlation coefficients of peak densities of protein profiles of *W. recta*, *W. curva*, *C. concisus*, *E. corrodens*, and *B. gracilis*.

TABLE 2. Mean inter- and intraspecies similarities of *Wolinella* species, *C. concisus*, *B. gracilis*, *Bacteroides ureolyticus*, and *E. corrodens*[a]

Taxon[b] (no. of strains)	Level of similarity (mean correlation coefficient ± SD)						
	W. recta	*B. gracilis*	*B. ureolyticus*	*W. succinogenes*	*C. concisus*	*W. curva*	*E. corrodens*
W. recta (4)	0.79 ± 0.06						
B. gracilis (4)	0.54 ± 0.06	0.83 ± 0.08					
B. ureolyticus (2)	0.49 ± 0.03	0.55 ± 0.04	0.76				
W. succinogenes (1)	0.56 ± 0.08	0.65 ± 0.01	0.53 ± 0.07	1.00			
C. concisus (3)	0.59 ± 0.08	0.58 ± 0.02	0.62 ± 0.06	0.71 ± 0.04	0.89 ± 0.01		
W. curva (3)	0.60 ± 0.05	0.56 ± 0.03	0.63 ± 0.11	0.60 ± 0.03	0.67 ± 0.03	0.75 ± 0.07	
E. corrodens (15)	0.51 ± 0.04	0.55 ± 0.03	0.59 ± 0.07	0.59 ± 0.03	0.61 ± 0.05	0.55 ± 0.05	0.76 ± 0.08

[a] Determined from correlation coefficients of densitometric scans of protein profiles. Gels were prepared from a single batch of acrylamide and run on the same electrophoretic run.
[b] The order of the species is based on cluster analysis.

TABLE 3. Mean inter- and intraspecies similarities of *Wolinella* species, *C. concisus*, *B. gracilis*, and *E. corrodens*[a]

Taxon[b] (no. of strains)	Level of similarity (mean correlation coefficient ± SD)				
	W. recta	*W. curva*	*C. concisus*	*E. corrodens*	*B. gracilis*
W. recta (4)[c]	0.73 ± 0.09				
W. curva (3)	0.60 ± 0.05	0.75 ± 0.07			
C. concisus (3)	0.60 ± 0.08	0.65 ± 0.04	0.83 ± 0.07		
E. corrodens (15)	0.50 ± 0.05	0.53 ± 0.06	0.59 ± 0.04	0.72 ± 0.11	
B. gracilis (4)	0.53 ± 0.05	0.57 ± 0.04	0.59 ± 0.03	0.56 ± 0.04	0.82 ± 0.07

[a] Determined from correlation coefficients of densitometric scans of protein profiles. Gels were prepared from two batches of acrylamide, and different electrophoretic runs were compared.
[b] The order of the species is based on cluster analysis.
[c] Same strains in duplicate runs for all species.

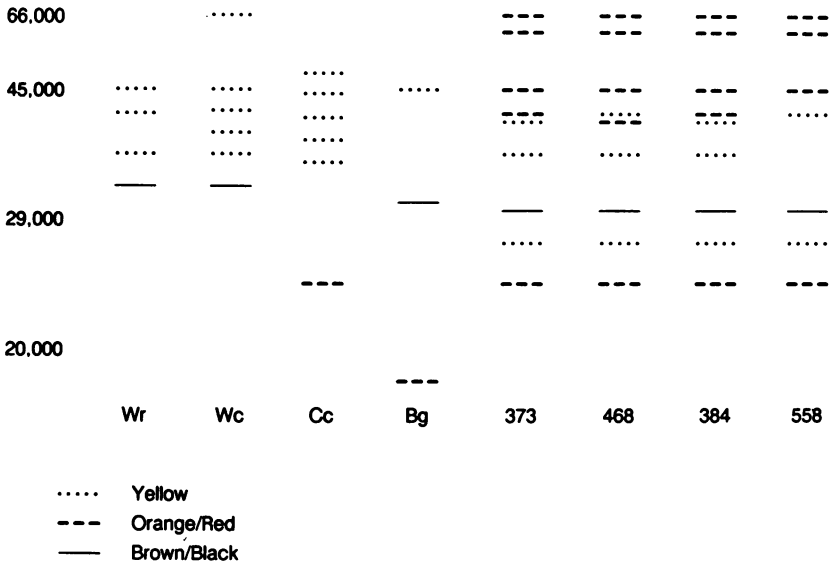

FIG. 3. Diagram of a gel stained with the colored silver stain (Gelcode) illustrating the positions of colored bands differentiating *W. recta* (Wr), *W. curva* (Wc), *C. concisus* (Cc), *B. gracilis* (Bg), and *E. corrodens* (373, 468, 384, and 558). With this stain, protein profiles of *E. corrodens* 373 and 384 were similar to each other but different from those of *E. corrodens* 468 and 558. Numbers at the left represent daltons.

should allow the investigation of the relationship between subgroups of this species and periodontal disease. Perhaps the enigma of finding this species elevated in proportions in progressing periodontal lesions of some subjects and arrested lesions of others (2) may be resolved by relating the subgroups of this species to the different clinical states.

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