Antigenic Relationships among *Bacteroides* Species Studied by Rocket-Line Immuno-electrophoresis

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Antigenic relationships among the 10 *Bacteroides* species, *Porphyromonas gingivalis*, *Prevotella bivia*, and *Prevotella intermedia* were investigated by rocket-line immuno-electrophoresis, using antigenic extracts prepared from type strains and antisera to *Bacteroides fragilis*, *Bacteroides distasonis*, *Bacteroides thetaiotaomicron*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. Among the members of the genus *Bacteroides*, *B. fragilis*, *Bacteroides caccae*, *Bacteroides stercoris*, *Bacteroides vulgatus*, *B. thetaiotaomicron*, and *Bacteroides ovatus* were found to be closely related, and *B. distasonis* and *Bacteroides merdae* were the species that were most distantly related to *B. fragilis*; *Bacteroides eggerthii* and *Bacteroides uniformis* were in an intermediate position. *Porphyromonas gingivalis*, *Prevotella bivia*, and *Prevotella intermedia* shared very low percentages of common antigens with the *Bacteroides* species. Detection of enzymic activities in immunoprecipitates allowed us to identify some antigens to enzymes and to study the immunological similarities of the enzymes. The malate dehydrogenases of the *Bacteroides* species were genus specific, and the glucose-6-phosphate dehydrogenases of most species exhibited antigenic similarities. The esterases of *B. distasonis* and *B. thetaiotaomicron* appeared to be species specific, whereas the esterases and phosphatases of *B. fragilis* cross-reacted with the esterases and phosphatases of *B. stercoris* and *B. vulgatus*, as did the phosphatases of *B. distasonis* and *B. merdae*. The phosphatases of *B. eggerthii*, *B. uniformis*, *Porphyromonas gingivalis*, and *Prevotella intermedia* were antigenically similar.

Organisms belonging to the genus *Bacteroides* Castellani and Chalmers 1919 are obligately anaerobic, gram-negative, nonsporing, rod-shaped bacteria (7, 8). However, this genus is known to be phenotypically and phylogenetically extremely heterogeneous. Thus, the 39 species included in the genus in *Bergey's Manual of Systematic Bacteriology* (7) exhibit a variety of cellular morphologies and saccharolytic and proteolytic activities and have DNA base compositions ranging from 28 to 61 mol% guanine plus cytosine.

On the basis of biochemical, chemical, and molecular biological criteria (10, 18, 20), the majority of the more typical *Bacteroides* species form three broad groups. The first group consists of *Bacteroides fragilis* and related saccharolytic intestinal species. This group includes five species which at one time were described as subspecies of a single species, *B. fragilis* (8) (*B. fragilis*, *Bacteroides vulgatus*, *Bacteroides ovatus*, *Bacteroides distasonis*, and *Bacteroides thetaiotaomicron*), and five species which either have been recognized more recently or have been considered similar to *B. fragilis* (*Bacteroides eggerthii*, *Bacteroides uniformis*, *Bacteroides caccae*, *Bacteroides merdae*, and *Bacteroides stercoris*) (10, 11). The second group comprises a large number of moderately saccharolytic taxa, such as *Bacteroides melaninogenicus* and related species, including *Bacteroides bivia* and *Bacteroides intermedius*. The third group includes the asaccharolytic pigmented species *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, and *Bacteroides endodontalis*. Recently, Shah and Collins (20) have proposed that the genus *Bacteroides* should be restricted to the 10 species belonging to the *B. fragilis* group and that the members of the second and third groups should be transferred to the new genera *Prevotella* and *Porphyromonas*,

respectively (19, 21). Several other new genera have been created (20) to accommodate many of the species formerly placed in the genus *Bacteroides* (7).

Previous immunological studies (1, 2, 16) have demonstrated the antigenic complexity and heterogeneity of the genus *Bacteroides*. However, Rogemond and Guinet (17) have investigated the antigenic relationships among five species belonging to the *B. fragilis* group (*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus*) by using cross immuno-electrophoresis (CIE) and have shown that some of the antigens with enzymic activities are species or group specific. The purposes of this study were to further examine the antigenic relationships among the 10 species currently placed in the genus *Bacteroides* and to compare these species with species that are now excluded from the genus by using another immunochemical technique, rocket-line immuno-electrophoresis (RLIE) (14, 15).

MATERIALS AND METHODS

Strains. The 14 bacterial strains used in this study and their sources are shown in Table 1; 13 of the isolates are the type strains (7) of the 10 *Bacteroides* species, *Porphyromonas gingivalis*, *Prevotella bivia*, and *Prevotella intermedia*. *B. fragilis* IPL E323 was included as representative of serotype E2 since *B. fragilis* type strain NCTC 9343 belongs to serotype E1 according to the serological classification of Beerens et al. (2, 4).

Antigenic extracts. Strains were grown anaerobically for 72 h at 37°C in TGY broth (Institut Pasteur Production) supplemented with a kanamycin-vancomycin mixture (Bio-Merieux, Craponne, France), vitamin K₁ (0.1 µg/ml), and hemin (10 µg/ml). Cells were harvested by centrifugation at 10,000 × g for 15 min. The resulting pellets were washed three times and resuspended in physiological saline. The cell suspensions were shaken for 2 min at 2,800 rpm in a Braun homogenizer by using 70-ml flasks (Braun Melsungen) filled

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TABLE 1. Cross-reactions among *Bacteroides*, *Porphyromonas gingivalis*, *Prevotella bivia*, and *Prevotella intermedia* antigens as determined by RLIE

Antigen	Protein concn (g/liter)	% of common antigens with:				
		<i>B. fragilis</i> serotype E1 antiserum	<i>B. distasonis</i> antiserum	<i>B. thetaiotaomicron</i> antiserum	<i>Porphyromonas gingivalis</i> antiserum	<i>Prevotella intermedia</i> antiserum
<i>B. fragilis</i> serotype E1 strain NCTC 9343	8	100 (19) ^a	53	83	3	21
<i>B. fragilis</i> serotype E2 strain IPL E323	12	100	53	83	3	21
<i>B. caccae</i> ATCC 43185	11	68	29	94	3	16
<i>B. distasonis</i> ATCC 8503	14	47	100 (17)	44	13	16
<i>B. eggerthii</i> ATCC 27754	16	47	35	44	7	11
<i>B. merdae</i> ATCC 43184	9	16	65	61	7	26
<i>B. ovatus</i> ATCC 8483	11	42	29	77	0	16
<i>B. stercoris</i> ATCC 43183	12	84	47	94	7	26
<i>B. thetaiotaomicron</i> ATCC 29148	12	47	29	100 (18)	0	21
<i>B. uniformis</i> ATCC 8492	8	53	41	67	7	21
<i>B. vulgatus</i> ATCC 8482	10	74	41	67	3	21
<i>Porphyromonas gingivalis</i> ATCC 33277	12	26	41	22	100 (30)	11
<i>Prevotella bivia</i> ATCC 29303	10	5	0	11	0	26
<i>Prevotella intermedia</i> ATCC 25611	6	21	18	22	3	100 (19)

^a The numbers in parentheses are the numbers of rockets in the homologous systems.

with 50 g of fine beads (diameter, 0.5 mm). The beads, cell debris, and unbroken cells were removed by centrifugation for 1 h at 20,000 × *g*, and the supernatants were freeze-dried and used for the study. Protein concentrations were estimated by the method of Lowry. Enzymes in antigenic extracts were detected either by zone electrophoresis in the first dimension (for glucose-6-phosphate dehydrogenase

[G6PDH] and malate dehydrogenase [MDH]) (17) or by the API-ZYM system (API System, La Balme les Grottes, France) (for alpha- and beta-esterases and phosphatase).

Antisera. Five polyclonal antisera were raised in rabbits against soluble extracts from *B. fragilis* serotype E1, *B. distasonis*, *B. thetaiotaomicron*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. The rabbits were immunized by

TABLE 2. Enzymes detected by RLIE

Antigen	<i>B. fragilis</i> serotype E1 antiserum				<i>B. distasonis</i> antiserum				<i>B. thetaiotaomicron</i> antiserum				<i>Porphyromonas gingivalis</i> antiserum				<i>Prevotella intermedia</i> antiserum			
	Alpha- and beta-esterases				Alpha- and beta-esterases				Alpha- and beta-esterases				Alpha- and beta-esterases				Alpha- and beta-esterases			
	Phosphatase	G6PDH	MDH		Phosphatase	G6PDH	MDH		Phosphatase	G6PDH	MDH		Phosphatase	G6PDH	MDH		Phosphatase	G6PDH	MDH	
<i>B. fragilis</i> serotype E1	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
<i>B. fragilis</i> serotype E2	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
<i>B. caccae</i>	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>B. distasonis</i>	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>B. eggerthii</i>	-	-	+	+	-	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-
<i>B. merdae</i>	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>B. ovatus</i>	-	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
<i>B. stercoris</i>	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
<i>B. thetaiotaomicron</i>	-	-	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-
<i>B. uniformis</i>	-	-	+	+	-	-	-	+	-	-	+	-	-	+	-	-	-	+	-	-
<i>B. vulgatus</i>	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>Porphyromonas gingivalis</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
<i>Prevotella bivia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Prevotella intermedia</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-

using an antigen-muramyl dipeptide mixture (6), and the titers of serum antibodies were evaluated by line immunoelectrophoresis (13).

RLIE. The RLIE procedure used was essentially the procedure described by Kroll (14, 15); a 0.8% agarose gel (standard low- M_r gel; Bio-Rad) and 0.025 M Tris buffer (pH 8.6) were used. Three gels were poured next to each other on GelBond sheets (Flobio, Courbevoie, France) from the cathode to the anode. One gel was 1.5 mm thick and contained holes (diameter, 2.5 mm) that were 10 mm apart for the antigenic extracts that were to be compared; the second gel was 1.3 mm thick and contained the antigenic extract used as a reference; and the third gel was 1 mm thick and contained the antiserum that was homologous to the reference antigen. The optimal antigen-antibody ratio was determined in preliminary line immunoelectrophoresis experiments (6). The concentrations of antigens and antisera in the last two gels varied from 5 to 20% (vol/vol). RLIE was performed at 2 V/cm for 18 h. Unprecipitated proteins were removed by pressing the gels and washing them in buffered saline for 1 h and in distilled water for 2 h. Then the gels were stained with Coomassie blue to detect all of the antigens and with various chromogenic substrates to detect the antigens with enzymic activities, as described previously (17). G6PDH, phosphatase, esterases, and MDH were detected successively. All of the antigenic extracts were tested against the different antisera at least 2 times and up to 12 times.

RESULTS

The cross-reactions among *Bacteroides*, *Porphyromonas gingivalis*, *Prevotella bivia*, and *Prevotella intermedia* antigens are shown in Fig. 1 and summarized in Table 1. The protein concentrations of the soluble antigenic extracts varied from 6 to 16 g/liter, which is within the acceptable range for immunoelectrophoretic studies (17). In general, the rocket patterns were highly reproducible. The average number of rockets obtained in the homologous reactions varied from 17 to 30. The numbers of rockets obtained in the heterologous reactions were counted, and the percentages of common antigens were evaluated by comparison with the corresponding homologous systems (defined as 100%).

MDH, esterases, and phosphatase were found in all antigenic extracts, whereas G6PDH was observed only in *Bacteroides* extracts. Cross-reactions among *Bacteroides*, *Porphyromonas gingivalis*, *Prevotella bivia*, and *Prevotella*

FIG. 1. RLIE pattern of *B. thetaiotaomicron* reference system obtained after Coomassie blue staining. Three gels were poured next to each other on GelBond sheets (Flobio) from the cathode to the anode. Gel I contained holes for the antigenic extracts to be compared; the wells were filled with 7- μ l portions of soluble extracts from *B. thetaiotaomicron* (well 1), *B. merdae* (well 2), *B. caccae* (well 3), *B. vulgatus* (well 4), *B. ovatus* (well 5), *B. fragilis* E2 (well 6), and *B. distasonis* (well 7). Gel II contained the antigenic extract used as the reference extract (Ag) (13 μ l of *B. thetaiotaomicron* soluble extract per cm^2 of gel). Gel III contained the antiserum homologous to the reference antigen (10 μ l of rabbit antiserum against *B. thetaiotaomicron* extract per cm^2). After RLIE was performed, the rockets standing on precipitin lines expressed related antigens for the reference antigen and the other antigenic extracts.

intermedia antigens with enzymic activities are shown in Table 2 and Fig. 2. Particular enzymic activities were not detected in the precipitates obtained with four homologous systems; G6PDH was absent from *B. distasonis*, phos-

TABLE 3. Differentiation of the type strains of *Bacteroides* species on the basis of antigenic enzyme patterns determined by RLIE

Antigen	<i>B. fragilis</i> serotype E1 antiserum		<i>B. distasonis</i> antiserum		<i>B. thetaiotaomicron</i> antiserum: alpha- and beta-esterases	<i>Porphyromonas gingivalis</i> or <i>Prevotella intermedia</i> antiserum: phosphatase
	Alpha- and beta-esterases or phosphatase	G6PDH	Alpha- and beta-esterases	Phosphatase		
<i>B. fragilis</i>	+	+	-	-	-	-
<i>B. caccae</i>	-	-	-	-	-	-
<i>B. distasonis</i>	-	-	+	+	-	-
<i>B. eggerthii</i>	-	+	-	-	-	+
<i>B. merdae</i>	-	-	-	+	-	-
<i>B. ovatus</i>	-	+	-	-	-	-
<i>B. stercoris</i>	+	+	-	-	-	-
<i>B. thetaiotaomicron</i>	-	+	-	-	+	-
<i>B. uniformis</i>	-	+	-	-	-	+
<i>B. vulgatus</i>	+	-	-	-	-	-

FIG. 2. Enzyme staining of the RLIE pattern of the *B. distasonis* reference system. Gel I contained wells that were filled with 7- μ l portions of soluble extracts from *B. stercoris* (well 1), *B. merdae* (well 2), *B. fragilis* E2 (well 3), *B. distasonis* (well 4), and *B. caccae* (well 5). Gel II contained the antigenic extract used as the reference antigen (Ag) (13 μ l of *B. distasonis* soluble extract per cm² of gel). Gel III contained the antiserum homologous to the reference antigen (10 μ l of rabbit antiserum against *B. distasonis* extract per cm²). After RLIE was performed, the gel was stained with various chromogenic substrates to detect phosphatase (P), alpha-esterase (E α), beta-esterase (E β), and MDH enzymic activities, giving specifically colored precipitin lines. The rockets standing on the precipitin lines expressed related antigens within the enzymic activities. Alpha-esterase and beta-esterase of *B. distasonis* are species-specific antigens since the corresponding rockets are present only in front of well 4. Two rockets derived from *B. merdae* (well 2) and *B. distasonis* (well 4) are present on the precipitate line corresponding to phosphatase, suggesting that the two antigens are related. Similarly, MDH rockets are present in front of all of the soluble extracts tested, indicating that the corresponding antigens are identical.

phatase and MDH were absent from *B. thetaiotaomicron*, esterases were absent from *Porphyromonas gingivalis*, and MDH was absent from *Prevotella intermedia*.

Table 3 shows the differentiation of the type strains of the 10 *Bacteroides* species on the basis of three enzymic activities (esterases, phosphatase, and G6PDH) and four antisera (anti-*B. fragilis*, anti-*B. distasonis*, anti-*B. thetaiotaomicron*, and anti-*Porphyromonas gingivalis* or anti-*Prevotella intermedia* antisera).

DISCUSSION

Because of the diversity of the organisms included in the genus *Bacteroides*, many techniques have been used to clarify the taxonomy of the genus. Knivett et al. (12) have established a numerical classification of the genus *Bacte-*

roides based on physiological properties, and Johnson (9) and Johnson and Harich (10) have categorized these organisms according to their levels of nucleic acid homology. Rogemond and Guinet (17) have compared the antigens and enzymes of *B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, and *B. vulgatus* by using CIE and *B. fragilis* and *B. distasonis* antisera.

In this study, we investigated the antigenic relationships among the 10 *Bacteroides* species, *Porphyromonas gingivalis*, *Prevotella bivia*, and *Prevotella intermedia* by using *B. fragilis*, *B. distasonis*, *B. thetaiotaomicron*, *Porphyromonas gingivalis*, and *Prevotella intermedia* antisera. RLIE allowed us to compare more antigenic extracts on one slide than CIE. With regard to the total antigens, RLIE performed with the homologous systems verified that the *Bacteroides*, *Porphyromonas*, and *Prevotella* species are antigenically complex, since 17 to 30 rockets were detected. However, still more antigenic precipitates were identified for *Bacteroides* spp. by CIE (17). The results of RLIE performed with the heterologous systems confirmed the antigenic similarities and dissimilarities among species. The two *B. fragilis* strains used (serotype E1 and E2 strains) were found to be 100% similar and gave identical percentages of common antigens with all antisera. The RLIE patterns of *B. fragilis*, *B. caccae*, *B. stercoris*, *B. vulgatus*, *B. thetaiotaomicron*, and *B. ovatus* were very similar, and *B. distasonis* and *B. merdae* appeared to be the species that are most distantly related to *B. fragilis*; *B. eggerthii* and *B. uniformis* were in an intermediate position. The percentages of common antigens detected by RLIE and CIE (17) for the same pairs of *Bacteroides* extracts and antisera were somewhat different, probably because of differences in the techniques and antigen or antiserum titers used. The strains of *Porphyromonas gingivalis*, *Prevotella bivia*, and *Prevotella intermedia* tested exhibited very low percentages of common antigens with each other and, as expected, with the *Bacteroides* species.

Previously published data (9, 10, 12, 17) and data from this study support the existence of at least the following three major clusters in the genus *Bacteroides*: (i) *B. fragilis* and *B. thetaiotaomicron*; (ii) *B. eggerthii* and *B. uniformis*; and (iii) *B. distasonis*. When *B. caccae* was studied (10; this paper), it was found to be similar to *B. fragilis*, and *B. merdae* was found to be similar to *B. distasonis*. The taxonomic position of *B. ovatus* and *B. vulgatus* is variable, depending on the authors. *B. ovatus* and *B. vulgatus* had more antigens in common with *B. fragilis* in RLIE gels than in CIE gels. In contrast to the data of Johnson and Harich (10), we found that *B. stercoris* was more closely related to *B. fragilis* than to the *B. eggerthii*-*B. uniformis* cluster. Our results confirm that the group formerly designated the *B. fragilis* group is a homogeneous collection of distinct species (7) that includes, among others, the three recently recognized taxa *B. caccae*, *B. merdae*, and *B. stercoris* (10, 11). Our findings support the proposals that the genus *Bacteroides* should be confined to these organisms (20) and that *Porphyromonas gingivalis* (19), *Prevotella bivia*, and *Prevotella intermedia* (21) should be placed in separate genera.

The presence and absence of key enzymes are characteristics that have value in microbial classification. Shah and Collins (18, 21) have shown that *Bacteroides* spp. differ from *Porphyromonas* and *Prevotella* spp. by possessing enzymes of the pentose phosphate pathway, such as G6PDH, whereas all three genera have MDH activity. All of the species tested in this study produce esterases and phosphatase, as revealed by the API-ZYM system. The presence and absence of

enzymes in our antigenic extracts were in complete agreement with the data of Shah and Collins (18, 21).

Immunoenzymic patterns, which consist of a few easily characterized precipitates, could be useful identification tools (17). However, as previously observed (17), although some enzymic activities are present in antigenic extracts, they could not be detected in the precipitates obtained with homologous systems. The reason for these discrepancies could be either a lack of the corresponding antibodies in antisera or the neutralizing properties of the antibodies. Whatever the cause, negative reactions cannot be interpreted when homologous reactions are unavailable or negative.

According to our results and as far as we could detect, the MDH of the *Bacteroides* species were all antigenically similar and quite different from the MDH of *Porphyromonas gingivalis*. The G6PDH of *B. fragilis*, *B. thetaiotaomicron*, *B. eggerthii*, *B. uniformis*, *B. ovatus*, and *B. stercoris* exhibited antigenic similarities. The esterases of *B. distasonis*, *B. thetaiotaomicron*, and *Prevotella intermedia* appeared to be species specific. The esterases and phosphatases of *B. fragilis*, *B. stercoris*, and *B. vulgatus* exhibited antigenic similarities, as did the phosphatases of *B. distasonis* and *B. merdae*. The phosphatases of *B. eggerthii*, *B. uniformis*, *Porphyromonas gingivalis*, and *Prevotella intermedia* were antigenically similar. Most of the results obtained by RLIE were consistent with the CIE results (17); the exceptions were the results for the esterases and/or the phosphatases of *B. ovatus* and *B. vulgatus*.

Bacteroides species are phenotypically very similar, and some of them are important human pathogens (5). Therefore, an identification scheme for these organisms based on simplified immunoenzymic patterns would be helpful, as suggested by Rogemond and Guinet (17). However, in our study, despite the fact that we used a higher number of antigenic extracts and antisera, it was still impossible to differentiate *B. stercoris* from *B. fragilis* and *B. eggerthii* from *B. uniformis*.

The use of monoclonal antisera to all of the species tested and the use of antigenic extracts from a larger set of strains should be helpful in further determining the antigenic relationships among *Bacteroides*, *Porphyromonas*, and *Prevotella* species.

REFERENCES

- Babb, J. L., and C. S. Cummins. 1981. Relationships between serological groups and deoxyribonucleic acid homology groups in *Bacteroides fragilis* and related species. *J. Clin. Microbiol.* **13**:369-379.
- Beerens, H., P. Wattré, T. Shinjo, and C. Romond. 1971. Premiers résultats d'un essai de classification sérologique de 131 souches de *Bacteroides* du groupe *fragilis*. *Ann. Inst. Pasteur (Paris)* **121**:187-198.
- Cato, E. P., and J. L. Johnson. 1976. Reinstatement of species rank for *Bacteroides fragilis*, *Bacteroides ovatus*, *Bacteroides distasonis*, *Bacteroides thetaiotaomicron*, and *Bacteroides vulgatus*: designation of neotype strains for *Bacteroides fragilis* (Veillon and Zuber) Castellani and Chalmers and *Bacteroides thetaiotaomicron* (Distaso) Castellani and Zuber. *Int. J. Syst. Bacteriol.* **26**:230-237.
- Cherniak, R., G. L. Lombard, and V. R. Dowell, Jr. 1979. Immunochemical evidence for multiple serotypes of *Bacteroides fragilis*. *J. Clin. Microbiol.* **9**:699-704.
- Duerden, B. I. 1983. The *Bacteroidaceae*: *Bacteroides*, *Fusobacterium*, and *Leptotrichia*, p. 114-136. In G. S. Wilson, A. A. Miles, and M. T. Parker (ed.), *Topley and Wilson's principles of bacteriology, virology and immunity*, 7th ed. Edward Arnold, London.
- Gabriel-Bruneau, S. M., and R. M. F. Guinet. 1984. Antigenic relationships among some *Candida* species studied by crossed-line immunoelectrophoresis: taxonomic significance. *Int. J. Syst. Bacteriol.* **34**:227-236.
- Holdeman, L. V., R. W. Kelley, and W. E. C. Moore. 1984. Genus I. *Bacteroides* Castellani and Chalmers 1919, p. 604-631. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
- Holdeman, L. V., and W. E. C. Moore. 1974. Genus I. *Bacteroides* Castellani and Chalmers 1919, p. 385-404. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Johnson, J. L. 1978. Taxonomy of the *Bacteroides*. I. DNA homologies among *Bacteroides fragilis* and other saccharolytic *Bacteroides* species. *Int. J. Syst. Bacteriol.* **28**:245-256.
- Johnson, J. L., and B. Harich. 1986. Ribosomal ribonucleic acid homology among species of the genus *Bacteroides*. *Int. J. Syst. Bacteriol.* **36**:71-79.
- Johnson, J. L., W. E. C. Moore, and L. V. H. Moore. 1986. *Bacteroides caccae* sp. nov., *Bacteroides merdae* sp. nov., and *Bacteroides stercoris* sp. nov. isolated from human feces. *Int. J. Syst. Bacteriol.* **36**:499-501.
- Knivett, V. A., H. N. Shah, A. S. McKee, and J. M. Hardie. 1983. Numerical taxonomy of some non-saccharolytic and saccharolytic *Bacteroides* species. *J. Appl. Bacteriol.* **55**:71-80.
- Kroll, J. 1973. Line immunoelectrophoresis, p. 61-67. In N. H. Axelsen, J. Kroll, and B. Weeke (ed.), *A manual of quantitative immunoelectrophoresis. Methods and application*. Blackwell Scientific Publications, Oslo.
- Kroll, J. 1973. Rocket-line immunoelectrophoresis, p. 83-87. In N. H. Axelsen, J. Kroll, and B. Weeke (ed.), *A manual of quantitative immunoelectrophoresis. Methods and application*. Blackwell Scientific Publications, Oslo.
- Kroll, J. 1983. Rocket-line immunoelectrophoresis. *Scand. J. Immunol. Suppl.* **10**:165-169.
- Lambe, D. W. 1980. Serology of *Bacteroidaceae*, p. 141-153. In D. W. Lambe, R. J. Genco, and K. J. Mayberry-Carson (ed.), *Anaerobic bacteria. Selected topics*. Plenum Press, New York.
- Rogemond, V., and R. M. F. Guinet. 1985. Antigens and enzymes of *Bacteroides* of the *Bacteroides fragilis* group compared by crossed immunoelectrophoresis. *Int. J. Syst. Bacteriol.* **35**:327-332.
- Shah, H. N., and M. D. Collins. 1983. A review: genus *Bacteroides*: a chemotaxonomical perspective. *J. Appl. Bacteriol.* **55**:403-416.
- Shah, H. N., and M. D. Collins. 1988. Proposal for reclassification of *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, and *Bacteroides endodontalis* in a new genus, *Porphyromonas*. *Int. J. Syst. Bacteriol.* **38**:128-131.
- Shah, H. N., and M. D. Collins. 1989. Proposal to restrict the genus *Bacteroides* (Castellani and Chalmers) to *Bacteroides fragilis* and closely related species. *Int. J. Syst. Bacteriol.* **39**:85-87.
- Shah, H. N., and M. D. Collins. 1990. *Prevotella*, a new genus to include *Bacteroides melaninogenicus* and related species formerly classified in the genus *Bacteroides*. *Int. J. Syst. Bacteriol.* **40**:205-208.