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Cell Wall Composition and Deoxyribonucleic Acid Similarities Among the Anaerobic Coryneforms, Classical Propionibacteria, and Strains of *Arachnia propionica*

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Eighty strains of anaerobic coryneforms were compared with 29 strains of classical propionibacteria and 8 strains of *Arachnia propionica* by cell wall analysis, deoxyribonucleic acid (DNA) base compositions, and nucleotide sequence similarities. The anaerobic coryneforms have DNA base compositions in the range of 58 to 64% guanine + cytosine (GC) and show at least three homology groups. The largest group corresponds to organisms identified as *Propionibacterium acnes* and shows about 50% homology to strains in the *P. avidum* homology group. The third group, *P. granulosum*, shows low levels of similarities to the other two. All strains of anaerobic coryneforms have some combination of galactose, glucose, or mannose as cell wall sugars, and most have alanine (ala), glutamic acid (glu), glycine (gly), and L-α-ϵ-diaminopimelic acid (L-DAP) as amino acids of peptidoglycan. However, a few strains in the *P. acnes* and *P. avidum* homology groups have *meso*-DAP and minimal amounts of glycine. Two serological types, based on cell wall antigens, were found in the *P. acnes* homology group. One type had galactose, glucose, and mannose as cell wall sugars, the other glucose and mannose only. The classical propionibacteria have DNA base compositions in the range of 65 to 68% GC and show four homology groups which correspond closely to van Niel's classification as given in the 7th edition of *Bergey's Manual*. The *P. jensenii* group showed about 50% homology to the *P. thoenii* group and about 30 to 35% to the *P. acidi-propionici* group. The *P. freudenreichii* strains showed a rather lower level of similarity (8 to 25%) to the other homology groups. Most of the strains of classical propionibacteria also have some combination of galactose, glucose, or mannose as cell wall sugars and ala, glu, gly, and L-DAP as peptidoglycan amino acids, but *P. shermanii* and *P. freudenreichii* strains, which form a single homology group, have galactose, mannose, and rhamnose as cell wall sugars and ala, glu, and *meso*-DAP in their peptidoglycan. There is a rather low level of DNA homology (10 to 20%) between the anaerobic coryneforms and classical propionibacteria. However, the strains of *A. propionica* which have a GC content of 64 to 65% and form a single homology group, show no homology to either of the other two major groups.

Propionic acid-producing bacteria were first described by von Freudenreich and Orla-Jensen in 1906 (22). The organisms were originally isolated from cheese, which also proved to be an ideal source for the isolation of additional types (63). In 1928 van Niel (68) placed the propionic acid-producing bacteria in a separate genus, *Propionibacterium* as suggested by Orla-Jensen (49), and designated eight species and one variety: *P. freudenreichii*, *P. pentosaceum*, *P. peterssonii*, *P. shermanii*, *P. rubrum*, *P. thoenii*, *P. technicum*, *P. jensenii*, and *P. jensenii* var. *raffinosaceum*. Subsequently, Werkman and Kendall (71) elevated *P. jensenii* var. *raffinosaceum* to the species level as *P. raffinosaceum*, while Hitchner (27,

28) proposed two more species, *P. zeae* and *P. arabinosum*. Sakaguchi et al. (58) proposed five additional species: *P. globosum*, *P. amylaceum*, *P. japonicum*, *P. orientum*, and *P. coloratum*, and one variety, *P. amylaceum* var. *aurantium;* and Janoschek (30) proposed three more species: *P. casei*, *P. pituitosum*, and *P. sangunieum*. All of these, and especially the eight species proposed by van Niel, are usually thought of as the "classical" propionibacteria.

The acne bacillus was described by Gilchrist in 1901 (24). Douglas and Gunter in 1946 (18), while comparing anaerobic coryneforms isolated from normal skin with authentic cultures of *Corynebacterium acnes*, observed that *C. acnes* produced propionic acid as a major acid product of glucose fermentation, and they proposed that the species be transferred to *Propionibacterium*. Similar findings were reported by Moore and Cato (43), who showed moreover that strains of *C. acnes* fermented lactate under properly reduced conditions. This finding supported the idea of the transfer, since ability to ferment lactate was part of the definition of the genus *Propionibacterium*.

At present, the taxonomy of the anaerobic, propionic acid-producing coryneforms is confused. Prevot et al. (54), using conventional biochemical tests including an examination of fermentation products, described eleven different species of anaerobic corynebacteria, *C. liquefaciens*, *C. adamsonii*, *C. parvum*, *C. avidum*, *C. renalecuniculi*, *C. diphtheroides*, *C. granulosum*, *C. anaerobium*, *C. lymphophilum*, *C. hepatodystrophicans*, and *C. pyogenes*. Prevot and Predette (53) added *C. acnes* to the 11 species listed above. It should be noted that the "*C. pyogenes*" of this list is "*C. pyogenes* bovis Roux 1905" which is not the beta-hemolytic organism common in suppurative conditions in animals and usually referred to as *C. pyogenes* (Glage) Eberson 1918. The 7th edition of *Bergey's Manual* (3) lists only five of these species, *C. acnes*, *C. parvum*, *C. granulosum*, *C. avidum*, and *C. diphtheroides*, and regards the *C. liquefaciens* of Prevot as identical to *C. acnes*.

Other investigators, e.g., Zierdt et al. (74) and Reid and Joya (57) have taken the opposite approach and have suggested that most or all of these species are synonymous with *C. acnes*. This extreme view was objected to by Moore et al. (45). Zierdt et al. (74), who examined representative strains of anaerobic coryneforms obtained from Prevot, found that all of them, except those labeled *C. granulosum* and *C. lymphophilum*, were lysed by bacteriophage 174, obtained from a strain of *C. acnes*.

In 1959, Pine and Hardin (52) isolated a facultative organism which morphologically resembled *Actinomyces israelii*, but which produced acetic and propionic acids as major products of glucose fermentation. Buchanan and Pine (5) further characterized the organism and named it *Actinomyces propionicus*. Gerencser and Slack (23) isolated additional strains. Recently Pine and Georg (50, 51) have reclassified *A. propionicus* and placed it in a new genus *Arachnia*, as *Arachnia propionica* in the family *Actinomycetaceae*. The relationship of this organism to the other propionic acid-producing bacteria is unclear.

This study was undertaken to see whether a combined study of cell wall composition and deoxyribonucleic acid (DNA) homology could shed light on the interrelationships of the "classical" propionibacteria, the anaerobic coryneforms, and strains of *A. propionica*. For comparison, several strains of aerobic coryneforms and other coryneform organisms whose DNA species have a guanine + cytosine content (% GC) in the range of 57 to 63% have also been examined. Preliminary accounts of some of these results have been published (11, 31).

MATERIALS AND METHODS

The origins and designations of the strains examined are listed in Table 1. For clarity, the strains are grouped according to the results of this study, but all strains have been checked to see if their nutritional and morphological characteristics fit the original descriptions.

The cultures were maintained on chopped-meat medium and were grown in peptone-yeast extract-glucose medium (PYG, reference 44) containing 0.1% Tween 80 (polyoxyethylene sorbitan mono-oleate), for inoculation into large cultures. Large cultures were grown in 3-liter Erlenmeyer flasks containing 2.5 liters of a medium consisting of mineral salts (44), 0.7% peptone (Difco), 0.3% Trypticase (BBL), 0.5% yeast extract (Difco), 1% glucose, 0.1% Tween 80, 0.05% cysteine, and 0.5% $NaHCO_3$. The methods for the preparation of 2.5-liter volumes of the anaerobic medium have been described previously (44). The cultures were incubated at 37 C with the exception of the *P. shermanii* and *freudenreichii* strains which were incubated at 25 C.

The above medium was modified for obtaining labeled DNA. The yeast extract and peptone were deleted, the Trypticase was increased to 1%, and ^3H-labeled adenine (250 μCi/200 ml, New England Nuclear) was added. A 5 to 10 ml culture, grown in PYG medium, was used as an inoculum.

Cell disruption. The centrifuged cells were suspended in 0.15 M NaCl, 0.01 M ethylenediaminetetraacetic acid (EDTA) buffer, *p*H 8.0 (40). Cells from 2.5-liter cultures could usually be suspended in 40 ml, although occasionally a greater volume was

TABLE 1. *Origins of the strains employed in the study*

VPI no.	ATCC no.	Source, other designations, and name[a]
		P. acnes type I
0020		Culture contaminant
0055		Culture contaminant
0121		Culture contaminant
0132		Culture contaminant
0156		A. R. Prévot 2201-A, *C. acnes*
0159	11827	ATCC
0163		A. R. Prévot 671, *C. anaerobium*
0164		A. R. Prévot 671, *C. anaerobium*
0199		A. R. Prévot 773, *C. liquefaciens*
0200		A. R. Prévot 814, *C. liquefaciens*
0207		A. R. Prévot 1383, *C. parvum*
0208		A. R. Prévot 2683, *C. parvum*
0272		Culture contaminant
0309		Culture contaminant
0389	6919	ATCC, NCTC 737, A. F. Hayden *C. acnes*, facial acnes
0781		Normal hog cecum
0846		Normal hog cecum
0985		Feces
1152-1		Turkey liver
1583		CDC 1343, synovial fluid
1596		CDC 1377, lymph node
1711-A		CDC 1461
1908		Culture contaminant
2418		A. R. Prévot 3518, *Actinobacterium naeslundii*
2518		H. P. Dalton, bone marrow
3230		H. P. Dalton M-1 53453, bone marrow of adult female
3243		A. Balows 31C
3320		A. R. Prévot 1321, *C. anaerobium*
3323		A. R. Prévot 1386, *C. granulosum*
3326		A. R. Prévot 4191, *C. granulosum*
3370		A. R. Prévot 3103, *C. anaerobium*
3706		Human feces
3712		Human feces
4167		Prévot 3183, *C. anaerobium*
4175		A. R. Prévot 1325, *C. diphtheroides*
4532		D. W. Lambe, 10R494, leg wound
4975		B. W. Lacey, Westminister Hospital, London
4978	6922	ATCC, NCTC 556, *C. acnes*
4979	11827	ATCC H. Seeliger strain Gerath
4980		Strain Baker, London Hospital, skin
4981		B. W. Lacey, Westminister Hospital, London
5047		London Hospital, normal skin
6672		CDC 554, clinical material
		P. acnes type II
0147		Culture contaminant
0162		A. R. Prévot 578, *C. anaerobium*
0174		A. R. Prévot 3723, *C. anaerobium*
0176		A. R. Prévot 355, *C. avidum*
0186		A. R. Prévot 2754 A, *C. diphtheroides*
0187		A. R. Prévot 2754 B, *C. diphtheroides*
0210	11829	ATCC, *C. parvum*
0391	11828	ATCC, *P. acnes*
0400-4		Culture contaminant
0429		Culture contaminant
0870		Hog large intestine
1127		Guinea pig liver
1199		Chicken gizzard
2123		Culture contaminant
4719		H. P. Dalton 86145, blood culture
0177-1		A. R. Prévot 355, *C. avidum*
3211-1		H. Beerens 452, *Corynebacterium* sp.
6673		CDC 604, clinical material
		P. avidum
0575		CDC 71, hip sinus
0576		CDC 811, submaxillary abscess
0589		CDC A518, wound
0668		CDC 3208 (1), chest pus
0671		CDC 7892
4160		A. R. Prévot 2068, *C. acnes*

[a] The first name is the person or institution from which the culture was received. A. R. Prévot, Institut Pasteur, Paris, France; H. P. Dalton, Medical College of Virginia, Richmond, Va.; A. Balows, Center for Disease Control, Atlanta, Ga.; D. W. Lambe, Emory University Hospital, Atlanta, Ga.; B. W. Lacey, Dept. of Bacteriology, Westminister Hospital, London, England; H. Beerens, Institut Pasteur, Lille, France; J. G. Voss, Research Division, The Proctor & Gamble Co., Cincinnati, Ohio; L. Pine, Center for Disease Control, Atlanta, Ga.; J. M. Slack, West Virginia Medical Center, Morgantown, West Va.; ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, Colindale, London, England; CDC, Center for Disease Control, Atlanta, Ga.; IAM, Institute of Applied Microbiology, University of Tokyo, Japan; VPI, Virginia Polytechnic Institute, Blacksburg, Va.

[b] May have been isolated from the same contaminated culture.

TABLE 1—*Continued*

VPI no.	ATCC no.	Source, other designations, and name[a]
4982		London Hospital, normal skin
		P. granulosum
0017		Culture contaminant
0506		Culture contaminant[b]
0507		Culture contaminant[b]
3334		A. R. Prévot 3999, *Eubacterium parvum*
4977		Strain Knight, London Hospital, acne lesion
5617		J. G. Voss, 9-1, *C. acnes* group II
5621		J. G. Voss, C-51, *C. acnes* group II
5622		J. G. Voss, D-21, *C. acnes* group II
5623		J. G. Voss, V-1, *C. acnes* group II
5625		J. G. Voss, 17-3, *C. acnes* group II
6500		A. R. Prévot 3085, *C. parvum*
		Other anaerobic coryneforms
0202		A. R. Prévot 1519 F, *C. lymphophilum*
0383		A. R. Prévot, SB, *Micrococcus saccharolyticus*
3413-1		V.P.I., skin
		P. freudenreichii
0392		IAM, Japan
0394	6207	ATCC; R, P. Tittsler, *P. freudenreichii*
0404	8262	ATCC; R. P. Tittsler (USDA) 62, *P. shermanii*
0405	9614	ATCC; R. J. Williams E11.1; C. B. van Niel, *P. shermanii*
0406	9614	ATCC; R. J. Williams E11.2; C. B. van Niel, *P. shermanii*
0407	9616	ATCC; R. J. Williams E11.3; C. B. van Niel, *P. shermanii*
0408	9617	ATCC; R. J. Williams E12.1; C. B. van Niel, *P. shermanii*
0409	13673	ATCC; Hoffman-La-Roche, Inc. 33, *P. shermanii*
5161	ISL101	ATCC; R. J. Williams E1.51, *P. freudenreichii*
5165	ISL105	ATCC; C. B. van Niel, 1.11, *P. shermanii*
		P. thoenii
0411	4872	ATCC; C. B. van Niel 19, *P. rubrum*
0412	4874	ATCC; C. B. van Niel 15, *P. thoenii*
5164	ISL104	ATCC; *P. rubrum*
5174	4874	ATCC; *P. thoenii* (0412)
		P. jensenii
0396	4867	ATCC; C. B. van Neil, 24, *P. jensenii*
0398	4869	ATCC; C. B. van Niel, 1, *P. jensenii*
0401	4871	ATCC; C. B. van Niel, 23, *P. rubrum*
0410	14073	ATCC; C. B. van Niel, E.6.1, C. H. Werkman, *P. technicum*
5162	ISL102	ATCC; C. B. van Niel, 1, *P. jensenii*
5163	ISL103	ATCC; C. B. van Niel, 29, *P. raffinosaceum* (5543)
5169	4870	ATCC; C. B. van Niel, 20, *P. peterssoni*
5173	4964	ATCC; E. R. Hitchner, *P. zeae*
0397	4868	ATCC; C. B. van Niel, 29, *P. jensenii*
5166	ISL106	ATCC; C. B. van Niel, 22, *P. technicum*
		P. acidi-propionici
0393	4965	ATCC; E. R. Hitchner, *P. arabinosum*
0399		Prévot, 14 X, *P. pentosaceum*, from Orla-Jensen
0402	4875	ATCC; C. B. van Niel, 4, *P. pentosaceum*
0403		IAM Japan, 1725, *P. shermanii*
5171	4965	ATCC, *P. arabinosum*
		A. propionica
0026	14157	L. Pine, CDC 699, *A. propionica*
	14157	ATCC; L. Pine, CDC 699
5068		J. M. Slack, 427
5069		J. M. Slack, 439 A
5070		J. M. Slack, 445
5072		J. M. Slack, 464
5074		J. M. Slack, 572

required. Twenty-milliliter amounts of 0.1-mm diameter glass beads and 20-ml volumes of cell suspension were placed in 50-ml bottles and shaken for 5 min at 4,000 cycles/min in a Braun mechanical cell homogenizer. The disrupted cells were separated from the glass beads by filtration through a no. 2 porosity sintered glass filter. Pronase (50 μg/ml, Calbiochem) was added to the cell material and incubated at 56 C for 1 to 2 hr. The disrupted cells were then centrifuged at 48,000 relative centrifugal force (RCF) for 20 min. DNA was isolated from the supernatant fluid, and cell walls were prepared from the pellet.

DNA isolation. To the supernatant fluid from the

disrupted cells was added 0.5% sodium lauryl sulfate and one-third volume of water-saturated chromatography grade liquid phenol. The mixture was shaken for 5 min, cooled, and centrifuged. The nucleic acids were precipitated from the aqueous phase with two volumes of 95% ethanol and collected by centrifugation. After dissolving the nucleic acids in 30 ml of 0.1× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0; reference 39) sufficient 20× SSC was added to bring the ionic strength to 1× SSC. The nucleic acids were then precipitated from the aqueous phase with two volumes of 2-ethoxyethanol and again collected by centrifugation. The precipitate was dissolved in 20 ml of 0.1× SSC. Six-inch lengths of dialysis tubing were knotted at one end, and the other end was slipped over an 18 by 100 mm plastic centrifuge tube which had its bottom cut out. The tubing was held on the tube by stretching a small rubber band around it several times. The dissolved nucleic acids were placed in the dialysis bags which were closed by placing rubber stoppers in the tubes. These were then suspended through holes in a Plexiglass plate into a 4-liter beaker containing 0.1× SSC. After dialyzing overnight, the bags were placed in test tubes (20 by 200 mm) containing 5 to 10 ml of NaCl-EDTA buffer. One milliliter of ribonuclease mixture (bovine pancreatic, 500 μg/ml, 5× crystallized, and T1, 25 units/ml, B grade; Calbiochem) was added to each nucleic acid preparation and incubated for several hours. After again dialyzing overnight, 0.5-ml amounts of the ribonuclease mixture were added as before and incubated for 3 to 4 hr; Pronase (50 μg/ml) was then added and the incubation was continued for a further 3 to 4 hr. After dialyzing overnight in another change of buffer, the nucleic acid preparations were removed from the bags and extracted several times with chloroform-octanol (39). After a final 2-ethoxyethanol and ethanol precipitation, preparations were obtained which contained 70 to 90% DNA. The amount of DNA was estimated by measuring the hyperchromic shift during thermal denaturation. A hyperchromic shift of 40% of the initial absorbance at 260 nm was taken as 100% DNA (17).

Per cent guanine + cytosine. The % GC of the DNA preparations was determined by their thermal melting point (T_m) (41) by using an automatic recording spectrophotometer (Gilford Instrument Laboratories). *Escherichia coli* B DNA was included in each spectrophotometer run as a standard. Although the DNA was fragmented to the extent that it was difficult to spool, the T_m was similar to that of DNA obtained from cells lysed by enzymes or detergent. Passage of a *Propionibacterium* DNA preparation through a French pressure cell at 0.9 to 1.0 kbar lowered the T_m by the same amount as did shearing a preparation of *E. coli* B DNA prepared from detergent-lysed cells.

DNA immobilization. Native DNA (50 μg/ml) was denatured by boiling for 5 min in 0.1× SSC and then quickly cooled by dilution to 5 μg/ml with cold 6× SSC. Denatured DNA was immobilized on 15-cm membrane filters (B6, Schleicher & Schuell) by slow filtration (25). After air-drying, the filters were placed in a 60 C oven overnight. The filters were stored at room temperature in a desiccator containing anhydrous $CaSO_4$. At the time of an experiment, filters (3 by 9 mm) were cut from a large filter with a Keysort card punch (no. 5203, McBee Systems). Each small filter contained 4 to 8 μg of DNA.

DNA duplex formation. Competition reactions were conducted using a variation of the Denhardt procedures (16, 32, 33). Ten microliters (0.5 to 1.0 μg of DNA) of labeled DNA fragments homologous to the DNA on the filter (reference DNA) and 100 μliters of 2.2× SSC or competitor DNA in 2.2× SSC were added to incubation vials (6 by 25 mm) (32). The vials were incubated for 15 hr at a temperature 25 C below the T_m of the reference DNA as determined in 1× SSC (40). The labeled DNA and competitor DNA preparations, dissolved in 0.1× SSC, were sheared to a size of about 500,000 daltons by two passages through a French pressure cell at 0.9 to 1 kbar (42) and denatured by heating in a boiling water bath for 5 min. In each competition experiment, the means of six replicates was used as the value for labeled DNA binding in the absence of competitor DNA, and the amount of binding was determined in duplicate and averaged for each level of competitor DNA. The homology values were calculated by dividing the depression in binding caused by 75 or 150 μg of heterologous competitor DNA by the depression resulting from 75 or 150 μg of the unlabeled homologous fragments and multiplying these ratios by 100. The homologous competitor DNA species depressed the binding by about 85% when 75 μg was used and by about 90% when 150 μg was employed. The per cent homology values were very similar for the two levels of competitor DNA, and so the averages of these values are recorded in the tables.

The incubation temperatures used with the various reference DNA species are listed in Table 2. The specific activity and the maximum homologous binding, determined when no competitor DNA was present, are also included for each of the ^3H-DNA preparations. The homologous binding values ranged from 26 to 44% and probably reflect varying amounts of ribosomal RNA contamination in the labeled DNA preparations and different amounts of unlabeled DNA fixed to the membrane filters. The nonspecific binding of labeled DNA to filters not containing DNA is negligible for all of the preparations (Table 2).

Cell wall analysis. The methods employed for the preparation of purified cell walls and for the determination of cell wall amino acids and sugars have been described (7).

Cell wall agglutination tests. Cell wall agglutination tests were done as previously described (9). The tests were incubated at 56 C for 2 hr and allowed to stand on the bench overnight before being read.

Preparation of antisera. Antisera were prepared by giving rabbits intravenous injections of partial disintegrates of the bacteria concerned, as described by Cummins (9).

Absorption of antisera. Absorption of antisera was done in two stages. The antisera were first absorbed at a dilution of 1/10, using 5 mg (dry weight) of cell walls for each milliliter of serum 1/10. When

TABLE 2. *Incubation temperatures, specific activities, and degree of nonspecific and homologous binding for the various reference DNA preparations*

VPI no.	Name	T_m	Incubation temp	Specific activity counts/min/μg	Per cent nonspecific binding	Per cent homologous binding
0389	*P. acnes* (type I)	93.5	68.5	4,460	0.05	36
0429	*P. acnes* (type II)	93.5	68.5	3,460	0.02	37
0575	*P. avidum*	94.6	69.5	3,930	0.02	40
0507	*P. granulosum*	95.3	70.5	2,860	0.50	28
0397	*P. jensenii*	96.5	71.5	7,600	0.17	33
0411	*P. thoenii*	96.3	71.5	4,360	0.15	44
0399	*P. acidi-propionici*	96.2	71.5	4,160	0.05	28
0407	*P. freudenreichii*	96.3	71.5	2,320	0.25	39
0202	*P. lymphophilum*	91.2	66.0	2,930	0.25	39
0026	*A. propionica*	95.5	70.5	13,000	0.13	26

necessary, the cell walls were thoroughly dispersed in the diluted serum with a tissue homogenizer. Absorption mixtures were placed in a 37 C incubator for 2 hr and were swirled gently by hand several times during this period. After this, they were generally left overnight at 4 C, and the serum was recovered by centrifuging at 20,000 RCF. The serum was then reabsorbed for 1 to 1.5 hr at 37 C with a further 5 mg of cell walls for each ml of serum 1/10, and the mixture was centrifuged again as above.

RESULTS

The strains employed in this study were originally received under a variety of specific names, but here they are placed in homology groups according to the nucleotide sequence similarities of their DNA species to DNA from various reference strains. We have given the homology groups specific names according to the species designation of the reference strain, although the status of these reference strains varies. In some cases, e.g., *P. acnes* 0389, *P. freudenreichii* 0407, and *A. propionica* 0026, neotype or type strains have been used (see Table 1 for details). In other cases, e.g., *P. granulosum* 0507 which was originally isolated as a contaminant of another culture, they have less respectable antecedents. However, in all cases the reference strains used had been examined phenotypically in the Anaerobe Laboratory and given provisional specific names as the results of these tests. Although they are not presented here, the phenotypic data (L. V. Holdeman and W. E. C. Moore, *personal communication*) have been used to compare the strains in the homology groups with the literature descriptions of the variously named species. When strains representing more than one correctly named species belong to the same homology group, the oldest or first listed name is used for that group.

Peptidoglycan amino acids. The peptidoglycan amino acid composition of the strains examined has not been investigated by quantitative methods. However, with certain interesting exceptions noted below, the qualitative two-dimensional paper chromatographic methods used show clearly that the peptidoglycan amino acids present in strains of all three major groups examined are alanine, glutamic acid, glycine, and L-diaminopimelic acid (DAP). These organisms thus provide another example of a group where L-DAP is regularly accompanied by glycine in peptidoglycan structure (cf. *Streptomyces*, reference 14). This is emphasized by the fact that in the unusual strains of *P. acnes* and *P. avidum* that have *meso*-DAP (*see below*) the glycine is minimal or absent, and this is true also of *P. freudenreichii* where the *meso*-isomer of DAP is found.

Our results on peptidoglycan amino acids thus agree with those of the more detailed quantitative studies of Schleifer et al. (60) on nine strains of classical propionibacteria: eight of these nine strains (all from the ATCC) were also examined in the present investigation.

The homology results, the patterns of cell wall sugars, and the results of cell wall agglutination tests are described separately for anaerobic coryneforms, classical propionibacteria, and *Arachnia propionica*, and the relationships between these three broad groups are considered in the Discussion section.

Anaerobic coryneforms. The data on these propionic acid-producing organisms, which have traditionally been placed in *Corynebacterium*, are shown in Table 3. The genus name *Propionibacterium* is used here. The three major homology groups which we have so far been able to distinguish are referred to as *P. acnes*, *P. avidum*, and *P. granulosum*.

(i) P. acnes homology group. This group is by far the largest, consisting of 65 strains with

TABLE 3. *Cell wall composition, serological reactions, and DNA similarities among the anaerobic coryneforms*

VPI no.	Wall composition				Antisera							Percent GC	Per cent homology to reference DNA			
					P. acnes				*P. granulosum*							
	DAP	Gal	Glu	Mann	Type I 3706	Type II 0162	3706 ABS 0162CW	0162 ABS 3706CW	0507	0507 ABS 3706	0507 ABS 0162		0389 *acne* I	0429 *acne* II	0575 *avidum*	0507 *granulosum*
P. acnes (type I)																
0020	L	+[a]	+	±	320[b]	160	160	–	20	–		59	95	95	48	
0055	L	+	+	±	320	160	160	–	20	–		59	98	100	59	
0121	L	+	+	±	320	160	160	–	–	–		58	100		51	
0132	L	+	+	±	320	160	160	–	±20	–		58	98	98		
0156	L	+	+	±	640	320	160	–	20	–		59	97	98	49	8
0159	L	+	+	±	320	80	160	–				59	95	100	52	8
0163	L	+	+	±	320	160	160	–	–	–		59	99			
0164	L	+	+	±	320	160	160	–				59	100	97	52	
0199	L	+	+	±	320	160	160	–				58	98	99	49	
0200	L	+	+	±	640	320	160	–				58	100	96	50	
0207	L	+	+	±	160	160	160	–				58	95	94	51	18
0208	L	+	+	±	160	80	160	–				59	100	100	51	
0272	L	+	+	±	320	80	160	–					98	100	50	
0309	L	+	+	±	320	40	160	–				58	98			
0389	L	+	+	±	320	160	160	–				59	100	94	52	20
0781	L	+	+	±	640	80	160	–				59	100		43	
0846	L	+	+	±	—[c]	—	—	—				59	86	92	52	
0985	L	+	+	±	320	80	160	–				58	100	93	48	
1152-1	L	+	+	±	160	80	160	–				58	97	100	50	
1583	L	+	+	±	160	80	160	–					97	99	52	13
1596	L	+	+	±	320	160	160	–					86			
1711-A	L	+	+	±	320	80	160	–					85	94	49	12
1908	L	+	+	±	320	80	160	–				58	96	99	56	17
2418	L	+	+	±	320	80	160	–				59	100	100	53	
2518	L	+	+	±	160	80	160	–				59	97	97	53	
3230	L	+	+	±	320	160	160	–				59	100		51	
3243	L	+	+	±	160	80	160	–					98	97	57	24
3320	L	+	+	±	320	80	160	–				57	93	92	47	
3323	L	+	+	±	320	80	160	–				58				

[a] The relative intensities of sugar spots in paper chromatograms are indicated by +++, ++, +, ±, tr (trace), or f. tr (faint trace).

[b] Figures are reciprocals of the highest dilution at which complete agglutination occurred; in some cases partial agglutination at low titer is indicated, e.g., ±20 means no agglutination at dilution of 1/20.

[c] For unexplained reasons, this cell wall preparation was autoagglutinable; the other results are typical of *P. acnes* type I.

TABLE 3—*Continued*

VPI no.	Wall composition				Antisera							Per cent GC	Per cent homology to reference DNA			
					P. acnes				*P. granulosum*							
	DAP	Gal	Glu	Mann	Type I 3706	Type II 0162	3706 ABS 0162CW	0162 ABS 3706CW	0507	0507 ABS 3706	0507 ABS 0162		0389 *acne* I	0429 *acne* II	0575 *avidum*	0507 *granulosum*
3326	L	+	+	±	640	320	160	–				59	97	96	57	
3370	L	+	+	±	320	80	160	–				58	99	98	51	
3706	L	+	+	±	320	160	320	–	40	–		59	97			
3712	L	+	+	±	320	80	160	–				59	97	93	49	
4167	L	+	+	±	320	160	160	–				60	99	100	46	
4175	L	+	+	±	160	80	160	–				60	93	99	55	18
4532	L	+	+	±	320	80	160	–					89	94		
4975	L	+	+	±	320	160	160	–				58	98	100	52	
4978	L	+	+	+	320	80	160	–				58	98	95	54	
4979	L	+	+	±	320	160	160	–				57	100	100	47	
4980	L	+	+	±	160	80	160	–				59	94	98	52	
4981	L	+	+	±	320	160	160	–				59	99	100	45	
5047	L	++	++	±	160	40	80	–								
6672	L	++	+	±								59	94	93	54	
***P. acnes* (type II)**																
0147	L	–	+	±	160	320	–	320	–			59				
0162	L	–	+	±	160	320	–	320	–			59	99		54	
0174	*meso*	–	+	±	80	160	–	80	–			59	90	93		
0176	L	–	+	±	160	320	–	160				60	90	98	50	
0186	L	–	+	±	40	320	–	160						93	49	
0187	L	–	+	±	80	160	–	160				59	91	96	52	
0210	L	–	+	±	40	320	–	320	–			59		98		
0391	L	–	+	±									94	90	57	
0400-4	*meso*	–	+	±	40	320	–	160	–			58	91			
0429	L	–	+	±	80	320	–	160				59	91	100	55	18
0870	L	–	+	±	40	160	–	160				59	88	97	50	22
1127	L	–	+	±	160	320	–	160				60	91	95	52	
1199	L	–	+	±	160	320	–	160					87	95	51	16
2123	L	–	+	±	80	320	–	160					89	95	49	9
2857-A	L	–	+	±	40	160	–	160					90	94	53	
4719	L	–	+	±	80	160	–	160				60	93	89	51	18
0177-1	L	–	+	±	80	320	–	160				60	87	92	55	
3211-1	L	–	+	±	40	160	–	160				60	88	95	49	12
6673	L	–	+	±								59	88	95	53	

TABLE 3—*Continued*

VPI no.	Wall composition				Antisera							Per cent GC	Per cent homology to reference DNA			
					P. acnes				*P. granulosum*							
	DAP	Gal	Glu	Mann	Type I 3706	Type II 0162	3706 ABS 0162CW	0162 ABS 3706CW	0507	0507 ABS 3706	0507 ABS 0162		0389 *acne*I	0429 *acne*II	0575 *avidum*	0507 *granulosum*
P. granulosum																
0017	L	++	+	+	40	–	40	–	160	80	160	63	12	14	16	88
0507	L	++	–	+	–	–			320	160	320	63	14	20	11	100
3334	L	+++	tr	+	–	–			160	320	320		22			87
4977	L	++	+	±	40	–	40		40	320	320	63	10		18	86
5617	L	++	±	+	40	–			160	160	320					100
5621	L	+++	±	+	±20	–			320	320		61				100
5622	L	++	f. tr	+	±20	–			40	40	20	61				100
5623	L	++	f. tr	+	20	–			320	320	160	62				99
5625	L	+	+	+	20	–			640	320	160	61				100
6500	L	++	tr	±	–	40	40		640							
P. avidum																
0575	L	+	+	+	–	–			–			62	54	50	100	15
0576	L	+	+	+	40	±20	20		40			62	52	52	92	
0589	*meso*	–	+	+	–	–			–			63		51	85	19
0668	L	+	+	±	–	–			–			62		54	100	
0671	L	+	+	+	–	–			–			62			93	19
4160									±20			62	55			
4982	*meso*	–	+	+	–	–			–			63	43	53	83	12
Unidentified propioni bacterium																
3413-1												60	41	48	31	

DNA species having GC contents of 59 ± 1%. There were no clear differences among the strains of *P. acnes* as measured by the per cent homology to the two reference strains (0389 and 0429), and all strains showed a high degree of homology (86 to 100%) to these two reference DNA preparations. However, when the pattern of cell wall sugars and the results of cell wall agglutination tests were considered, the strains fell clearly into two groups, which we have referred to as *P. acnes* types I and II. The cell walls of the type I strains contain galactose, glucose, and mannose whereas those of type II strains contain glucose and mannose only. This difference is also clearly seen in the serological reactions of the two types of cell walls. There is considerable cross-reaction between the two types when unabsorbed antisera are used, although the titers against heterologous sera are almost always lower. However, when absorbed sera are used, the distinction between the two types is evident. It appears that the immunological specificity associated with the type I strains is due to galactose, since this sugar is not present in type II strains. On acid hydrolysis, galactose is rapidly released from the type I cell walls; it was the only hexose detected after 30 min of hydrolysis (2 N H_2SO_4, 100 C), which suggests it is an end-sugar and therefore likely to be important in determining antigenic specificity. In acid hydrolysates of type II walls examined at intervals up to 2 hr, glucose and mannose were released rather slowly and in approximately equal amounts.

Craddock (8) isolated 47 strains of the acne bacillus from acne pustules and comedones and noted two types of colony, one large, heaped up, and yellowish, and the other smaller and flatter. When antisera prepared against strains of each type were tested against cell suspensions, it appeared that the two types were antigenically distinct. Commercial antisera (Difco) are available against two strains of *P. acnes*, 554 and 604 (VPI no. 6672 and 6673). These two strains were originally isolated from clinical material at the Center for Disease Control, Atlanta, Ga. (V. R. Dowell and L. V. Holdeman, *personal communication*), but it is not known whether they correspond to the two types described by Craddock. It appears that most strains of *C. acnes* are agglutinated by one or the other of the two sera, and often by both. For example, Ray and Kellum (56) found this to be the case in slide agglutination tests with all of the 61 strains of presumed *P. acnes* that they tested against the two antisera. In terms of cell wall composition we have found strain 554 to be a typical type I *acnes*, and strain 605 to be a typical type II, but we have not made tests with absorbed antisera to determine whether the two types can in fact be recognized by slide agglutination.

(ii) P. avidum homology group. This consists of only seven strains (Table 3). The GC content of the DNA preparations is 62 to 63%, and all show high homology to reference DNA from strain 0575.

These *P. avidum* strains show some interesting relationships with the much larger *P. acnes* homology group. In the first place, in spite of a base composition difference of 3 to 5% GC, the DNA species of the *P. avidum* strains and *P. acnes* strains show about 50% homology. Secondly, two cell wall types are evident, which resemble the two types found in *P. acnes*. Four strains (0575, 0576, 0668, and 0671) have galactose, glucose, and mannose, whereas two strains (0589 and 4982) have only glucose and mannose. The resemblance is strengthened by the fact that both of the latter strains have *meso*-DAP instead of the L-isomer, and the only other group where this isomer was found was *P. acnes* type II (strains 0174 and 0400-4). These similarities in cell wall structure suggest that the cistrons responsible are conserved in these two groups of related organisms. However, there must be differences in detailed structure of the carbohydrates, since, in cell wall agglutination tests with *P. acnes* type I and type II antisera, only one *P. avidum* strain (0576) showed cross-agglutination, and that at a low titer. Unfortunately, a *P. avidum* serum was not available, but this group merits further investigation because of the rather clear division into two types and the similarities with *P. acnes*.

(iii) P. granulosum homology group. The strains in this group have DNA containing 62 to 63% GC. Although the % GC of the DNA is similar to that of the *P. avidum* group, the two groups show only 15 to 20% homology with each other (Table 3). The level of similarity between the *P. granulosum* and *P. acnes* group is of the same order.

There are obvious variations in the proportions of the three hexoses present in cell wall polysaccharides from the *P. granulosum* strains, and some, such as 0017 and 4977, show a considerable degree of cross-agglutination with *P. acnes* antiserum, while others, such as 0507, show none. However, cell wall suspensions from most strains agglutinated with an antiserum to 0507 (the DNA reference strain for the group), even after absorption with *P. acnes* type I or type II cell walls.

While this work was in progress, Voss (68) published the results of his examinations of

146 strains of *P. acnes* isolated from human skin and acne lesions. Voss was able to divide his strains into two groups on the basis of biochemical reactions, susceptibility to phage, and gel-diffusion analysis of soluble antigens in whole broth cultures. The DNA base compositions of three strains of each type showed that group I (the largest group, 120 strains) had a GC content of 59 to 61%, while group II (17 strains) had a GC content of 63 to 65%. Since the GC content of the group II strains suggested that they might belong in one or other of the groups which we had provisionally called the *P. avidum* or *P. granulosum* groups, J. G. Voss kindly sent us five of these strains for examination (VPI no. 5617, 5621, 5622, 5123, 5625). All of these were found to have high homology with the *P. granulosum* reference strain 0507, and also to belong to the *granulosum* group serologically (see Table 3).

The majority of the anaerobic coryneform strains that we examined fell into the three aforementioned homology groups. However, results with some other strains show that further groups of this kind undoubtedly exist, although so far we have not identified enough strains to define them fully. For example, strain 3413-1 was originally identified as *P. acnes* on the basis of phenotypic characteristics. The GC content of the DNA from this strain (60%) is characteristic of the *P. acnes* group, but the degree of homology to the *P. acnes* reference strain was 41 to 48% (Table 3). Although this suggested that the 3413-1 might be *P. avidum*, DNA from it showed only 31% homology with DNA of the *P. avidum* reference strain (0575); thus it appears that 3413-1 represents another homology group.

Two strains, one referred to us as *C. lymphophilum* (VPI no. 0202) and the other as *Micrococcus saccharolyticus* (VPI no. 0383), were quite distinct from all the other propionic acid-producing bacteria but appeared to be related to each other. The DNA species from these strains had a GC content of 53 to 54% and showed no homology with any of the other reference DNA preparations, but DNA from 0383 showed 62% homology to DNA from 0202. These two organisms had cell wall polysaccharides composed of galactose, glucose, and mannose, but had lysine instead of DAP, and their walls showed no serological reactions with any of the antisera used in the investigation.

Since many of the aerobic coryneform bacteria (64) and other anaerobic coryneforms have DNA species whose GC content is similar to that of the propionic acid-producing organisms, it was of interest to determine whether there were any nucleotide sequence similarities. DNA was isolated from several strains and tested against reference DNA from *P. lymphophilum* (0202), *P. acnes* (0389), and *P. avidum* (0575) (Table 4). Although the base compositions of the DNA species are similar, there was negligible homology with any of the reference strains.

Classical propionibacteria. Among the 29 strains of classical propionibacteria examined were representatives of 11 species described in the 7th edition of *Bergey's Manual* (3). Strains of the species described by Sakaguchi et al. (58) and Janoschek (30) were not available. The majority (24 strains) of the 29 strains examined showed a GC content of 65 to 67% with extremes of 64 to 68%. The strains could be arranged in four groups, each of which showed a high homology (80% or more) with one of the reference strains. (Table 5). These homology groups are referred to here by the specific names *P. jensenii*, *P. thoenii*, *P. acidi-propionici*, and *P. freudenreichii*.

The *P. jensenii* and *P. thoenii* homology groups showed about 50% DNA homology with each other, and the *P. acidi-propionici* homology group showed from 33 to 39% homology to the *P. jensenii* and *P. thoenii* reference strains. There is no characteristic pattern of cell wall components for these groups; all have L-DAP and some combination of galactose, glucose, and mannose. Indeed, the general pattern of components for all of these strains is remarkably similar to that found among the anaerobic coryneforms.

The strains in the *P. freudenreichii* homology group all show a high level of homology to the reference strain (0407) and only a low level of nucleotide similarity to the other groups of classical propionibacteria (Table 5). This homology group is also distinctive in two other ways: all of the strains in it have the *meso*-isomer of DAP, and all have a cell wall polysaccharide characterized by rhamnose, galactose, and mannose. This pattern of wall components had previously been found in another strain of *P. shermanii* (14). In the present investigation, no strains in any other group were found to have rhamnose, although in earlier work this sugar had been detected in the cell walls of one strain of *P. technicum* (NCIB 5965) and one of *P. jensenii* (NCIB 8069) (14). These two strains, however, had the L-isomer of DAP, thus differing from *P. shermanii*. We have in fact re-examined a different subculture of NCIB 8069, in the form of ATCC 4869, and did not find rhamnose in it. It is possible that accidental mislabeling of cultures was responsible for the original report of rhamnose.

TABLE 4. *Competition by DNA from other coryneform bacteria*

Strain	Per cent GC	Per cent homology to DNA from		
		P. lympho-philum 0202	*P. acnes* 0389	*P. avidum* 0575
Corynebacterium xerosis, C105 (NCTC 9735)	57	15	7	0
C. pyogenes, C100 (NCTC 5224)	58	0	3	0
C. pyogenes, C33 (637S; see reference 1)	58	0	0	0
C. pyogenes, ATCC 8104	58	0	0	0
Lipophilic coryneform, 002[a]	58	12	6	13
Lipophilic coryneform, 006[a]	58	0	7	0
Lipophilic coryneform, 007[a]	60	12	7	0
Lipophilic coryneform, 2-39[b]	57	3	3	0
Lipophilic coryneform, 5-17[b]	61		0	7
Actinomyces bovis, C35 (Leo Pine)	63	1	2	0
A. bovis, C274 (ATCC 13683)	63	0	13	0
Bifidobacterium bifidum, 0094[c]	60	1	9	2

[a] Culture Collection, Langley Field, Va.
[b] Culture Collection, R. F. Smith, Temple University, Philadelphia, Pa.
[c] Culture Collection, VPI.

Arachnia propionica. The third group of propionic acid-producing organisms which we have examined is represented by a collection of strains labeled *A. propionica*. This species has only fairly recently been distinguished from the morphologically very similar *Actinomyces israelii* (5, 51).

Two patterns of cell wall sugars were shown by these strains (Table 6): 0026, ATCC 14157, and 5068 have essentially only galactose, whereas the others have galactose, glucose, and a trace of mannose. The only antiserum available was one against ATCC 14157, and all strains except one (5072) cross-reacted with it to some degree, but the group is obviously not homogenous with respect to wall composition and needs to be investigated further. We were more interested in determining its degree of homology to other propionic acid-producing bacteria, and as far as this is concerned, the results are clear-cut (Table 6): the degree of DNA homology to any of the other groups is very low.

The last three cultures in Table 6 had been sent to the Anaerobe Laboratory as *A. propionica*, but they showed no homology with the reference DNA (0026). On further examination, it is obvious that one (5071) is a strain of type I *acnes*, while another (5073) has no DAP and on this ground alone could not be *A. propionica;* it is probably *Actinomyces naeslundii*. The third strain (5077) has not been identified. The results with these three strains have been included to emphasize the difficulties which may arise due to some combination of misidentification, contamination, or mislabeling in morphologically similar organisms.

DNA base compositions. The DNA base compositions of representative anaerobic coryneforms and classical propionibacteria have been determined by other workers (2, 61, 69). Our results when comparing the same strains are consistently 2 to 3% lower than those of Sebald et al. (61), who used chromatographic methods, and those of Voss (69), who used buoyant density (59). Bouisset et al. (2), who also used chromatographic methods, found the DNA species from strains of *C. acnes*, *C. avidum*, and *C. granulosum* to range from 48 to 52%; however, these figures are so low that it is difficult to reconcile them with the other data above.

DISCUSSION

There is a technical problem connected with the taxonomy of these organisms which must be mentioned at the outset, and this is that anaerobic coryneforms are not uncommon contaminants of anaerobic cultures. One of us (C.S.C.) has in the past used cultures labeled *Actinomyces israelii*, *A. bovis*, and *A. naeslundii* to prepare cell wall samples and was puzzled to find that all three contained large amounts of L-DAP, which was not present in the cell walls of other strains of these species. As judged by stained smears, the aberrant cultures appeared to be pure, but in retrospect it seems certain that the original organism had been replaced by an anaerobic coryneform of similar morphology. Beerens (1) has described a similar state of affairs in a study of six strains of nonsporulating anaerobic bacteria (*A. bovis*, two strains; *A. israelii; C. acnes; C. avidum;* and *C. liquefaciens*). At the time he

TABLE 5. *Cell wall compositions and DNA similarities among the classical propionibacteria*

VPI no.	Wall composition					Per cent GC	Per cent homology to reference DNA			
	DAP	Gal	Glu	Mann	Rham		0397	0411	0399	0407
P. jensenii										
0396	L	−	+	tr	−	66	87	52	32	13
0397	L	+	+	tr	−	66	100	53	28	12
0398	L	+	++	f. tr	−	68	87	53	33	11
0401	L	+	++	f. tr	−	66	84	49		7
0410	L	+	+	f. tr	−	66	87	53		
5162	L	±	+	−	−	65	86	48	28	
5163	L	+	+	±	−	66	99	49	33	6
5166	L	±	++	f. tr	−	68		51	33	
5169	L	+	++	f. tr	−	67	86	56	29	13
5173	L	+	++	f. tr	−	68	78	48	23	5
P. thoenii										
0411	L	+	+	±	−	66	54	100	30	12
0412	L	+	++	±	−	66	52	93	31	
5164	L	+	++	f. tr	−	66	51	98	27	10
5174	L	++	++	f. tr	−	67	54	93	32	11
P. acidi-propionici										
0393	L	++	++	−	−	67	36	35	82	
0399	L	+	+	f. tr.	−	66	38	36	100	8
0402	L	+	++	±	−	66	39		91	
0403	L	−	+	−	−	67	36	33	83	10
5171	L	−	++	f. tr	−	68	39	35	79	5
P. freudenreichii										
0392	*meso*	+++	−	±	++	67	19	20	24	91
0394	*meso*	+++	−	+	+	64	21	19	15	86
0404	*meso*	++	−	±	+	65	31		24	91
0405	*meso*	++	−	+	+	65	31	21		90
0406	*meso*	+++	−	++	++	65	22	20	27	85
0407	*meso*	+++	−	+	+	65	28	18	24	100
0408	*meso*	++	−	+	+	65	34	20	27	92
0409	*meso*	+++	−	++	+	65	23		33	92
5161	*meso*	+++	tr	++	+	67	25	25	24	89
5165	*meso*	+++	−	+	+	66	21	19		87

examined them, he found the properties of all six cultures to be identical with those of *C. acnes*. Linzenmeier (36, 37) has also pointed out how readily *C. acnes* can contaminate anaerobic cultures. He found that whereas suspensions of some strains of anaerobic coryneforms agglutinated only with homologous antisera, a considerable number of other strains all reacted equally well to several antisera including three made against strains of *C. acnes*. Some cultures of *A. bovis* and other microaerophilic actinomycetes reacted similarly to the *acnes* antisera. As a further example, we may point to the three strains, mentioned above, which had been referred to the Anaerobe Laboratory as *A. propionica*.

It may be added that this phenomenon is not confined to contamination of cultures with *P. acnes*, since some cultures which we have found to belong to other homology groups were originally isolated as contaminants. For example strain 0017 (*P. granulosum*) was isolated from a culture of *A. propionica* (0026), and 0400-4 (*P. acnes*) was isolated as a contaminant from a culture of *P. peterssonii* (ATCC 4870).

The source of this type of contamination of cultures of anaerobes is most likely to be scales from the skin or scalp of the person making the transfer. Since *P. acnes* is usually the predominant organism on the skin (21), contamination with it is likely to be the most frequent. Replacement of the original culture by a contaminant is relatively easy to detect when there are gross morphological differences between the two; however, where the organisms

TABLE 6. *Cell wall composition and DNA similarities among strains of Arachnia propionica*

Strain	Cell wall composition				Cell wall agglutination tests with antiserum to			Per cent GC	Per cent homology to reference DNA from	
	DAP	Gal	Glu	Mann	*A. propionica* ATCC 14157	*acnes* I 3706	*acnes* II 0162		0026	0389 (*acnes*)
0026[a]	L	+	–	–	640	±20	–	64	100	1
ATCC 14157	L	+	–	–	160	±20	–	65	95	
5068	L	+	Tr	–	640	±20	–	64	92	0
5069	L	±	±	Tr	40	–	–		89	
5072	L	±	±	Tr	–	–	–	65	89	0
5074	L	+	±	Tr	160	–	–	63	87	0
CDC W973	L	+	±	Tr	160				86	0
CDC W1054	L	+	±	Tr	160				86	
Received as *A. propionica*										
5067	L	++	Tr	+	–	–	–	64	1	
5071	L	+	+		–	160	80	59	6	100
5073[b]	None	Tr	+	+	–	–	40	65	0	

[a] 0026 and ATCC 14157 are two subcultures of the same strain.
[b] Cell walls of strain 5173 also contained rhamnose.

are morphologically similar it may be necessary to re-examine the culture in some detail to establish that contamination has occurred, unless some single test, such as change in DAP isomer, can be used. Unfortunately, there seems to be no reason why this type of contamination should not also affect cultures of the anaerobic coryneforms themselves, as they are handled in the laboratory, and this may have been a source of confusion in describing the properties of some species.

Another point which may have some bearing on the apparent discrepancies between the phenotypic characters reported from different laboratories is the growth-stimulating effect of oleic acid on these organisms. We have found Tween 80 to be stimulatory for both the anaerobic coryneforms and the classical propionibacteria, and Puhvel and Reisner (55) and Ray and Kellum (56) have noted a similar effect. It seems possible that fermentation tests performed in media deficient in oleic acid would give rise to false-negative results because of poor growth.

Broadly speaking, the strains of anaerobic coryneforms and the classical propionibacteria appear to form a rather homogeneous group as far as cell wall compositions are concerned, with certain interesting exceptions which are described in more detail below. The general pattern of components in the wall is some combination of glucose, galactose, and mannose as cell wall sugars, with alanine, glutamic acid, glycine, and L-DAP as the amino acids of peptidoglycan. These findings are in agreement with earlier findings on six strains of *P. acnes* (C. S. Cummins, J. Gen. Microbiol. **34**, 1964) and five of other propionibacteria (14); however, Werner and Mann (72) examined nine strains of *C. acnes* and *C. parvum* and reported that, as well as galactose, glucose, and mannose, the strains they examined contained fucose (and in one strain, xylose). Four of these nine strains (ATCC 11829, 6919, 6922, 11828) were examined in the present investigation, but we have not been able to detect the presence of any sugar other than glucose, galactose, and mannose in the hydrolysates of their cell walls.

The exceptions to the general pattern described above are twofold. First, all 10 strains of *P. shermanii* and *P. freudenreichii* had a distinctive pattern of cell wall sugars consisting of galactose, mannose, and rhamnose (Table 5), and all of them contained *meso*-DAP instead of the L-isomer. This is essentially the same pattern of components as that found previously in a single strain of *P. shermanii* (NCIB 5964; reference 14). The occurrence, within a genus, of a species which differs from the other species of the genus with respect to the peptidoglycan diamino acid has been noted elsewhere among gram-positive organisms. For example, in the genus *Bacillus*, *B. spaericus* contains lysine in the vegetative cell wall instead of *meso*-DAP (29), and in *Lactobacillus*, *L. plantarum* contains *meso*-DAP instead of lysine (13). In *Clostridium*,

examples of both kinds of alteration occur; the majority of species contain *meso*-DAP, but *C. perfringens* has L-DAP, whereas members of some other species have lysine (15). The finding of an unusual isomer of DAP in strains of the *P. freudenreichii* group would not seem, therefore, to be any reason for replacing it in a separate genus. However, it is perhaps unfortunate that *P. freudenreichii* was designated as the type species, since some other species which contained L-DAP would be more representative of the genus *Propionibacterium* as a whole. *P. jensenii* would be a more logical choice because it contains L-DAP and shows nucleotide similarities with the majority of the classical propionibacteria.

The other exception to the general pattern of cell wall components was more unexpected. As can be seen in Table 3, certain strains in the *acnes* and *avidum* homology groups were found to have *meso*-DAP in place of L-DAP. These strains showed the same degree of homology (80 to 100%) to the reference DNA as did other strains in the same group, and did not appear to differ significantly in any other characters examined. Only four strains of this kind have so far been identified; two of these are *P. acnes* type II and two are in the *P. avidum* homology group. The mucopeptide amino acid pattern of these strains is also distinctive, since all four showed only trace amounts of glycine in place of the heavy glycine spot normally found in chromatograms of cell wall hydrolysates of strains which contain L-DAP. A further character that all four strains have in common is the complete absence of galactose from their cell walls. However, they are not unique in this respect, since galactose is absent from all strains of type II *acnes*.

These strains with *meso*-DAP do not appear to be very numerous among the anaerobic coryneforms, since they form only about 5% of the 90 or so strains in the homology groups *P. acnes*, *P. avidum*, and *P. granulosum*. However, their occurrence is of considerable interest, since it shows that it is no longer possible to assume that all strains allocated to a single species will have the same isomer of DAP.

In the present work we used cell wall agglutination tests initially to see whether the various homology groups of anaerobic coryneforms could be distinguished by particular cell wall antigens, and this appears to have been successful in the case of the *acnes* and *granulosum* groups. It is hoped that the method can be extended to other homology groups when more strains are available. We have not made any comparison between the results of cell wall agglutination tests and agglutination of suspensions of intact bacteria of the same strain. It has been argued previously (10) that it is not easy to compare the results of the two types of test, because new antigenic determinants are often exposed in preparing cell wall suspensions. Moreover, a considerable number of strains of anaerobic coryneforms are autoagglutinable (6, 36).

In the course of this work, antisera were also prepared against *P. jensenii*, *P. acidi-propionici*, *P. thoenii*, and *P. shermanii*. These sera gave only rather low titers (1/40 to 1/160) against cell wall suspensions of the homologous organisms, and the results have not been included in the tables. In general they were in agreement with the homology groupings, in that there was a considerable amount of cross-reaction between unabsorbed antisera and cell wall suspensions of *P. jensenii*, *P. acidi-propionici*, and *P. thoenii* but no cross-reaction between these three and strains of *P. shermanii* and *P. freudenreichii*. There was also a scattering of low-level cross-reactions between the cell walls of classical propionibacteria and antisera to *P. acnes* types I and II and *P. granulosum*.

It would appear that in this, as in other groups of bacteria, the principal use for serological analysis is to discover antigenic determinants unique to each homology group, so that newly isolated strains can be rapidly identified. It is assumed that the antigenic determinants of these cell wall preparations are the polysaccharides whose gross composition is shown by the sugars detected in cell wall hydrolysates. If this is so, it is not surprising to find serological cross-reactions between different groups, since all the anaerobic coryneforms and the classical propionibacteria except *P. shermanii* and *P. freudenreichii* have some combination of galactose, glucose, and mannose in their cell walls. We feel that these low-level cross-reactions can be ignored for taxonomic purposes, since the homology results give a far better overall measure of the relatedness of the various groups.

Taxonomic considerations: anaerobic coryneforms. The taxonomy of these organisms has been complicated in the past because all strains are morphologically extremely similar, and because many species have been inadequately described. As we have suggested above, these difficulties may also have been made worse by the problems of unrecognized contamination, and perhaps by the use of media containing suboptimal amounts of oleic acid.

The species *C. acnes* and *C. liquefaciens* have been considered identical for some time

(3). Zierdt et al. (74) have proposed that additional species, *C. anaerobium*, *C. pyogenes* (bovis), *C. granulosum*, *C. parvum*, and *C. diphtheroides*, also be considered synonymous with *C. acnes*. Reid and Joya (57) supported these conclusions and in addition regarded *C. avidum*, *C. lymphophilum*, and *C. pyogenes* as variants of *C. acnes*. Our results are in agreement with some of the above considerations but not all. Strains that were labeled and fit the descriptions of *C. liquefaciens*, *C. anaerobium*, *C. adamsonii*, and *C. diphtheroides* were found to belong to our *P. acnes* types I and II. On the other hand, organisms fitting the original description of *P. avidum* (19) form a somewhat homogeneous homology group that is distinct from *P. acnes* although they have greater similarity to it than to other homology groups (Table 3).

The organisms that we place in the *P. granulosum* homology group for the most part fit the phenotypic description of *P. granulosum* (34, 53, 54). Brzin (4) suggested two groups within *C. acnes*, and Voss (69) has extended the characterization of these groups, one of which he referred to tentatively as *C. acnes* type II. These strains could be distinguished from typical *C. acnes* strains by the fact that the large majority of them did not hydrolyze gelatin, produce indole, or reduce nitrates and because they fermented maltose, sucrose, melezitose, and trehalose, which the typical *C. acnes* strains did not. We have found that Voss's *C. acnes* type II strains also belong to the *P. granulosum* homology group.

Whether or not there is an organism that should be referred to as *P. parvum* is not clear. Our study included ATCC 11829 (VPI 0210), the *C. parvum* strain employed by Reid and Joya (57), and results of both laboratories agree that it is a strain of *C. acnes*. One of Prevot's strains of *C. parvum* (3085) was used by both Zierdt et al. (74) and by us (VPI 6500). Zierdt and his co-workers found the culture to resemble *C. acnes*, whereas in our hands it belonged to the *P. granulosum* homology group.

We have used absorbed sera for *P. acnes* types I and II and *P. granulosum* to screen other strains labeled *C. parvum*, and out of 50 such strains have found only 4 which appear to be neither *acnes* nor *granulosum*. Until these strains have been studied in greater detail, we prefer to leave open the question of whether or not there exists a group of strains which can be differentiated sufficiently well to justify calling it *P. parvum*. At the moment it appears from our results that most strains labeled *parvum* are *acnes* type I or II.

C. pyogenes (bovis) [not *C. pyogenes* (Glage) Eberson 1918; see Zierdt (73)] was regarded by Zierdt et al. (73, 74) as being the same as *C. acnes*. Reid and Joya (57) agreed with Zierdt and noted the fact that *C. pyogenes* ATCC 8104 is catalase-positive. However, ATCC 8104, although weakly catalase-positive (12), is otherwise typical of the beta-hemolytic animal pathogen *C. pyogenes* (Glage) Eberson 1918, which differs in many respects from anaerobic coryneforms. Our cultures of *C. pyogenes*, including ATCC 8104, show no DNA homology with the *P. acnes* or *P. avidum* reference strains (Table 4). Strains labeled *C. pyogenes* (bovis) were not included in our study.

Reference organism 0202 was isolated by Prevot and was designated *C. lymphophilum* (54) since it appeared to fit the description given by Torry (66). This isolate (Prevot no. 1519F) has been the only one studied by Prevot et al. (54), and many of the characteristics attributed to *C. lymphophilum* were determined in this strain. The phenotypic characteristics of the organism as determined in the Anaerobe Laboratory were very similar to those found by Prevot (*personal communication*) so that it is almost certainly the same organism. The DNA of one other organism, VPI 0383 (Prevot's strain SB), showed 62% homology to 0202 DNA. Although these organisms are quite distinct from the other propionic acid bacteria, it is suggested that the specific epithet *lymphophilum* be used for them and that for the present they be kept in the genus *Propionibacterium* at least until more strains have been more fully studied.

Classical propionibacteria. The classical propionibacteria are represented by four homology groups (Table 5). It is both interesting and instructive to compare these results with the classification of *Propionibacterium* by van Niel, as given in the 7th edition of *Bergey's Manual* (Table 7), especially since we have included a number of van Niel's original strains in our investigations (see Table 1). If the arrangement of species advocated by van Niel is compared with the homology group data, it is evident that the agreement between the two is extremely good. Only 3 strains out of 26 were in anomalous positions, and two of these, *P. arabinosum* (0392) and *P. shermanii* (0403), were received in the Anaerobe Laboratory at the same time and the labels may have been reversed. Moreover, the intergroup relationships are clearly reflected in the figures for per cent homology. For example, the relative isolation of the *P. freudenreichii* group from the others is quite clear, as is the fact that sub-

TABLE 7. *Comparison between Van Niel's classification and homology groups*

Van Niel's classification (*Bergey's Manual*, 7th ed.)	Homology group data		Per cent homology to reference DNA from[a]			
	Provisional name of group	Original species designation	*P. freudenreichii* 0407	*P. thoenii* 0411	*P. jensenii* 0397	*P. acidipropionici* 0399
Group I						
P. freudenreichii	*P. freudenreichii*	*P. shermanii* (7)[b]	90	20	26	25
P. shermanii		*P. freudenreichii* (2)				
		P. arabinosum (1)[c]				
Group II, A						
(i) *P. rubrum*	*P. thoenii*	*P. rubrum* (2)	12	96	53	30
P. thoenii		*P. thoenii* (1)				
(ii) *P. zeae*	*P. jensenii*	*P. zeae* (1)	17	51	88	30
P. technicum		*P. technicum* (2)				
P. raffinosaceum		*P. raffinosaceum* (1)				
P. peterssonii		*P. peterssonii* (1)				
P. jensenii		*P. jensenii* (3)				
		P. rubrum (1)[c]				
Group II, B						
P. arabinosum	*P. acidi-propionici*	*P. arabinosum* (2)	8	35	38	87
P. pentosaceum		*P. pentosaceum* (2)				
		P. shermanii (1)[c]				

[a] Figures taken from Table 8.
[b] Number of strains with given designation.
[c] Strains which do not agree with Van Niel's classification.

groups (i) and (ii) of group II A in van Niel's classification show about 50% homology with each other, whereas the degree of homology between either of them and strains in group II B is rather lower (30 to 38%).

Since the figures for per cent homology are an overall estimate of the degree of relatedness, it would seem that van Niel's classification is soundly based, even though it originally depended on the results of relatively few tests. However, it might be preferable to reduce the number of species names to four, corresponding to the number of homology groups and, if necessary, designate varieties to take account of the variation in fermentation patterns within the groups. The homology group data may also be compared with those of Malik et al. (38), who used numerical taxonomy to study a group of 56 strains of *Propionibacterium*, representing 8 species. The agreement between the two sets of results is good, and both point to the very close relationship between strains of *shermanii* and *freudenreichii* and between *pentosaceum* and *arabinosum*. The main point of divergence concerns the relationship between *P. rubrum* and *P. thoenii*, since Malik et al. (38) found their three strains of *rubrum* to show only a low level of similarity with three strains of *thoenii*, while we found two strains of *rubrum* and one of *thoenii* to show high homology. However, the number of strains in both investigations is small, and it seems likely that examination of a larger group of brown- or red-pigmented strains would establish their relationships more clearly.

Genus designation. As shown by our use of genus designations throughout this paper, we agree with Douglas and Gunther (18) and others (26, 43) that the anaerobic coryneforms should be placed in *Propionibacterium*. On the basis of gross cell morphology, these organisms could be placed in either *Propionibacterium* or *Corynebacterium*. However, the absence of arabinose and the presence of L-DAP in the cell walls, the anaerobic character, and the production of propionic acid as the major acid of fermentation all indicate a relationship to the propionibacteria rather than to the human and animal coryneforms of the type of *C. diphtheriae*. In the character of the cell lipids also, the anaerobic coryneforms appear to be distinct from the corynebacteria sensu stricto, since they do not contain the corynemycolic and corynemycolenic acids (C_{32} to C_{36}) which seem to be characteristic of the human and animal pathogenic coryneforms (20, 35, 62, 70). The principal fatty acids in the anaerobic coryneforms and the classical propionibacteria are C_{15} branched-chain compounds (46–48).

TABLE 8. *Intergroup DNA similarities*

Homology group and reference strain	Per cent GC	Per cent homology to reference strains							
		0389	0575	0507	0397	0411	0399	0407	0026
P. acnes (0389)	57-60[a]	97[b]	51	16	1	8	10	1	3
P. avidum (0575)	62-63	50	90	17	10	11	8	4	1
P. granulosum (0507)	62-64	12	15	95	14	8	7	4	1
P. jensenii (0397)	66-67	4	18	11	88	51	30	17	0
P. thoenii (0411)	66-67	8	20	16	53	96	30	12	0
P. acidi-propionici (0399)	66-68	8	15	16	38	35	87	8	0
P. freudenreichii (0407)	64-67	5	16	16	26	20	25	90	5
A. propionica (0026)	64-65	1	8	3	2	4	5	6	90

[a] The range of % GC determinations within the group.

[b] The figures given for the homologous groups are averages of values shown in Tables 3, 5, and 6. The intergroup values were obtained from heterologous competition experiments employing several DNA preparations.

The intergroup DNA competition experiments suggest some DNA similarities between the anaerobic coryneforms and the classical propionibacteria. This is especially the case when reference DNA species from *P. avidum* and *P. granulosum* were tested against DNA from the classical propionibacteria (Table 8). However, the homology levels do not rise above 20% and are more often about 10 to 15%. With reference DNA from *P. acnes* 0389 the levels are still lower, probably reflecting the greater difference in base composition between the preparations (*P. acnes* 57 to 60%; classical propionibacteria 64 to 68%). These results are near the level of experimental error and one must interpret them with caution. However, in most cases the degree of homology is consistently above the level found with presumably unrelated organisms such as *C. xerosis* and *C. pyogenes* (see Table 4).

The phylogenetic position of strains of *Arachnia propionica* is difficult to determine. Morphologically these strains are very similar to *Actinomyces israelii* and they occur in similar ecological situations. However, in the amino acid pattern of their peptidoglycan and in the production of propionic acid they resemble the propionic acid bacteria. The results of the present study show that the *A. propionica* strains all have high homology to the reference strains for the group (0026) and low homology (1 to 6%) for both the anaerobic coryneforms and the classical corynebacteria. In this respect they are clearly distinct from either of the other two major groups we have studied. However, similar low homologies were found between some of the anaerobic coryneforms and the classical propionibacteria (see Table 8).

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