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Differentiation of Two Groups of *Corynebacterium acnes*

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One hundred and forty-three strains of *Corynebacterium acnes*, isolated from human skin and acne lesions, were compared with three strains of *Propionibacterium acnes* from the American Type Culture Collection. The 146 organisms could be separated into two groups. Members of the larger group (129 strains) hydrolyzed gelatin and usually produced indole, Gel-In(+), but were unable to ferment trehalose, maltose, or sucrose, TMS(−). The deoxyribonucleic acid from selected strains of this group had an average guanosine + cytosine (GC) content of 60.5%. The members of the smaller group (17) were Gel-In(−), TMS(+), and the deoxyribonucleic acid had an average GC content of 63.9%. Studies with absorbed and unabsorbed antisera to the smaller group showed that although there were antigens shared by the two groups, it was possible to distinguish them serologically. Members of each group produced propionic acid. The principal fatty acid component of members of each group was iso-C_{15} fatty acid. Seventy per cent of the Gel-In(+) strains were lysed by phage 174, whereas only one of 15 Gel-In(−) strains was lysed. Pending further information on the genetics of the two groups, those Gel-In(−), TMS(+) strains are tentatively designated *C. acnes*, group II.

There exists considerable confusion over the taxonomy of the anaerobic corynebacteria, and particularly of *Corynebacterium acnes*. Douglas and Gunter (7) placed this species in the genus *Propionibacterium* because of its fermentative production of propionic acid, although more recent opinion appears to favor its retention in *Corynebacterium* (13, 17, 22). There has been widespread disagreement concerning its biochemical reactions, although four recent descriptions (4, 13, 17, 22) of the species have been reasonably consistent. Only Brzin (4) recognized the possible presence of two types, of which one produced indole and reduced nitrate but failed to ferment some sugars; these reactions were reversed in the other type. Salient features of these four descriptions, as well as of those by Breed et al. (2) and Prévot (16), are summarized in Table 1.

Our studies on *C. acnes* isolated from normal human skin and from acne lesions confirmed the distinction seen by Brzin (4), and applied the additional criteria of phage susceptibility, antigenic specificity, and deoxyribonucleic acid (DNA) composition to the clarification of the relationship of these organisms.

MATERIALS AND METHODS

Cultures. Strains 6919, 6921, and 11827 were obtained from the American Type Culture Collection as *P. acnes*. An additional 143 strains were isolated from the surface of normal human skin or from acne lesions. Isolations were made, and stock cultures were grown on Brain Heart Infusion Agar (Difco) with 1% added glucose, incubated anaerobically at 37 C in an atmosphere of 95% N_2 and 5% CO_2.

Biochemical methods. Gelatin hydrolysis was determined by liquefaction of 12% gelatin in Trypticase Soy Broth (BBL) over a period of a month, or by streaking plates containing 0.4% gelatin, 1% tryptone (Difco), 0.1% Yeast Extract (Difco), and 1.5% agar and flooding with acidic $HgCl_2$ (8) after 1 week. Indole production was demonstrated after 4 days of growth in broth containing 2% tryptone and 0.1% Yeast Extract. Nitrate reduction was determined after 4 days of growth in Brain Heart Infusion Broth plus 0.5% glucose and 0.02% KNO_3; zinc dust was added to negative reactions to assure the continued presence of nitrate. Sugar fermentations were tested after addition of 10% solutions of filter-sterilized sugars to C T A (Cystine Trypticase Agar) Medium (BBL) to a sugar concentration of 1%; tubes were incubated for a minimum of 2 weeks. For demonstration of lipolysis, plates containing 1% triglyceride emulsified by ultrasonic treatment in Trypticase Soy Agar were streaked with the test organism and observed after 1 week for an area of clearing adjacent to the zone of growth.

Fatty acid determinations. The volatile fatty acids produced by fermentation of 2% glucose in a medium consisting of 1% tryptone, 0.1% Yeast Extract, and 0.1% KH_2PO_4 at *p*H 6.8 for 1 week were separated

TABLE 1. *Salient points of descriptions of Corynebacterium acnes*

Character	Breed et al. (2)	Prévot (16)	Brzin (4)		Moss et al. (13)	Puhvel (17)	Zierdt et al. (22)
Gelatin	+	±	+	+	+	+	+
Indole	+	+	+	−	65%[a]	85%	+
$NO_3 \rightarrow NO_2$	+	+	+	−	+	76%	+
Maltose	+	+	−	+	−	76%	ND
Sucrose	(+)[b]	+	−	+	−	−	−
Melezitose	ND[c]	ND	−	+	ND	ND	ND
Dextrin	ND	ND	−	+	ND	ND	ND
Trehalose	ND	ND	+	+	ND	+	+
Lysis by phage 174	ND	ND	ND	ND	ND	ND	+

[a] Per cent positive.
[b] Slight.
[c] Not done.

from clarified medium (15) by steam distillation, titrated and corrected for the HCl distilled, and identified by gas chromatography. An F & M 402 chromatograph was used with a 1-m glass column (4 mm, inner diameter) packed with 10% FFAP (Varian-Aerograph) on 100/120 mesh Gas Chrom Q. After a 10-min initial delay, column temperature was raised from 130 to 180 C at a rate of 3 C per min.

Because of the possible taxonomic significance of the branched-chain C_{15} fatty acids (12, 13), cells of six strains were saponified with 15% KOH in 50% methanol under N_2, and the fatty acids were extracted for preparation of the methyl esters (14). Esters were separated at 180 C on a Perkin-Elmer 226 gas chromatograph with a 50-m stainless steel capillary column (0.25 mm, inner diameter) coated with polyphenyl ether. Reference peaks were obtained with *n*-C_{15}, iso-C_{15}, and anteiso-C_{15} (Applied Science Laboratories) methyl esters for identification of the peaks in the cell extracts.

Lysis by phage. Appropriate dilutions of phage 174 (22), obtained from C. H. Zierdt (National Institutes of Health), were dropped on bacterial lawns prepared on Brain Heart Infusion-glucose agar, and the plates were observed after 2 days for lysis. In many cases, phage 26 (from S. M. Puhvel, University of California, Los Angeles) was also used.

Precipitin tests. Antisera against selected strains were prepared in rabbits by injection of a mixture of killed bacterial cells and Freund's complete adjuvant (Difco) into the foot pads; after a month, a series of four intravenous injections of killed cell suspensions was made. The rabbits were bled 10 days after the last injection. Double-diffusion precipitin tests were carried out in barbital buffer (6.98 g of sodium barbital and 6.0 g of NaCl per liter, *p*H 7.4; reference 6) containing 1% Ionagar No. 2 (Colab), by using whole broth cultures as antigens and undiluted antisera. Reactions were carried out at room temperature.

RESULTS

All strains were catalase-positive, gram-positive, unevenly stained, pleomorphic rods, measuring about 1.5 by 0.6 μm in size. Biochemical reactions and susceptibility to phage suggested that the 146 strains could be divided into two groups as indicated in Table 2. Group I corresponds most closely to the organism as described by Breed et al. (2) and Prévot (16) and by most previous workers, particularly in gelatin liquefaction, indole production, and nitrate reduction; however, these strains consistently failed to ferment maltose, sucrose, trehalose, and melezitose. All of the ATCC strains belonged to this group. Group II strains, on the other hand, failed to liquefy gelatin or produce indole, and few reduced nitrate; all strains fermented some or all of the sugars mentioned above. These differences are similar to those observed by Brzin (4), with the exception that we failed to observe fermentation of dextrin by the group II strains and of trehalose by group I strains. As reported by Zierdt et al. (22), a majority of the group I strains were lysed by phage 174; susceptibility to phage 174 was closely paralleled by susceptibility to Puhvel's phage 26. Of the group II strains, only one was lysed by the two phages.

Growth on agar containing 1% tributyrin or triolein showed that representative strains of both groups were able to hydrolyze both triglycerides, with some variation in size of the area of hydrolysis.

Eleven strains of each group were examined for the nature of the volatile acids produced by fermentation of glucose. The average production of volatile fatty acids by group I strains was 1.78 meq/100 ml, whereas that of group II strains was only 0.38 meq/100 ml. In all cases, propionic acid was the only compound produced which could be detected by gas chromatography; there

TABLE 2. *Physiological characteristics observed with Corynebacterium acnes groups I and II*

Character	Group I[a]	Group II[a]
Gelatin	129/129	0/17
Indole	119/129	0/17
$NO_3 \rightarrow NO_2$	8/11	2/15
Maltose	0/129	15/17
Sucrose	0/129	12/17
Melezitose	0/13	10/15
Dextrin	0/13	0/15
Trehalose	0/13	14/15
Lysis by phage 174	75/106	1/15

[a] Number positive to number tested.

was a net loss of the small amount of acetate originally present in the medium.

Determination of the nature of the C_{15} fatty acids which were reported to be abundant in the cell lipids of *C. acnes* (12, 13) was carried out on the three group I strains from the ATCC and on three group II strains. In all cases, as shown in Fig. 1, the principal fatty acid component of the cell lipids was identified as the iso-C_{15} acid (13-methyl tetradecanoic acid).

After preparation of antisera to several strains, it was found that the two groups could be readily distinguished by sera prepared against group II strain D-34. Figure 2 shows the nonidentity of reactions of pooled antiserum in the center well with the homologous strain D-34 and with group I cultures 6919, 6921, 11827, and 174, in agar-diffusion precipitin tests. Complete absorption of the antiserum with cells of strain 6919 eliminated the reactions with group I cultures (Fig. 3). On the other hand, "reactions of identity" were

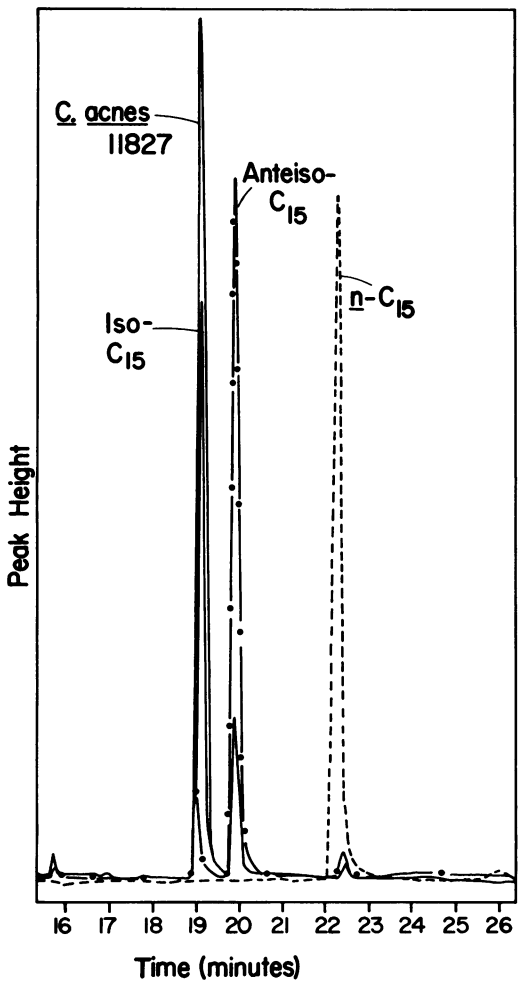

FIG. 1. *Gas chromatographic analysis of methyl esters of C_{15} fatty acids from C. acnes ATCC 11827 and n-, iso-, and anteiso-C_{15} fatty acids.*

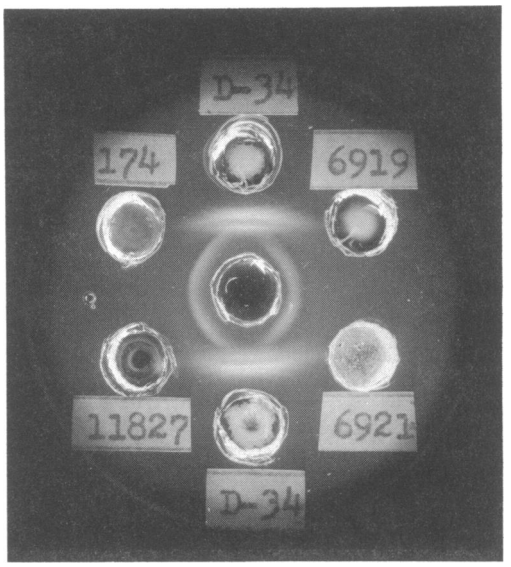

FIG. 2. *Agar-diffusion precipitin tests. Anti-D-34 (group II) serum in center well; D-34 (group II), 6919, 6921, 11827, and 174 (group I) cells in outer wells.*

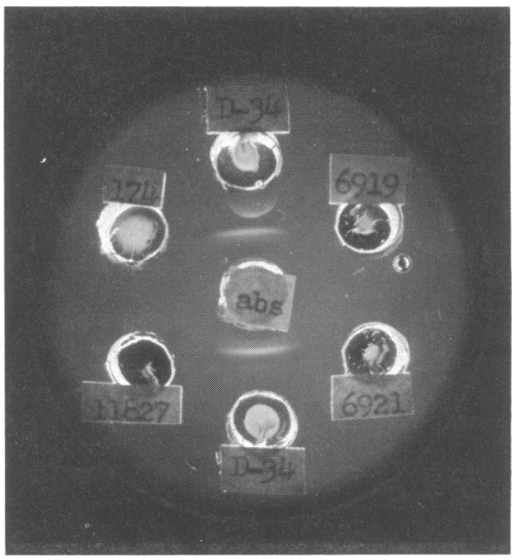

FIG. 3. *Agar-diffusion precipitin tests. Anti-D-34 (group II) serum absorbed with 6919 cells (group I) in center well; D-34 (group II), 6919, 6921, 11827, and 174 (group I) cells in outer wells.*

produced in precipitin tests with group II cultures D-34, V-1, C-51, C-54, and D-21 (Fig. 4), and were not affected by absorption of the antiserum with cells of group I strain 6919 (Fig. 5).

In addition, determinations of DNA base composition of three strains of each type were kindly performed by Manley Mandel (University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston) by buoyant density in CsCl gradients. Duplicate and triplicate determinations were carried out, with calculation of the guanosine plus cytosine (GC) content of the DNA; the results are summarized in Table 3, from which it is evident that %GC of group I strains is lower than that of group II. Mandel (*personal communication*) states that these values are accurate to within about 1%. In addition, he was able to resolve a mixture of DNA from strains 6919 and D-34 into two bands, further demonstrating nonidentity of the DNA species.

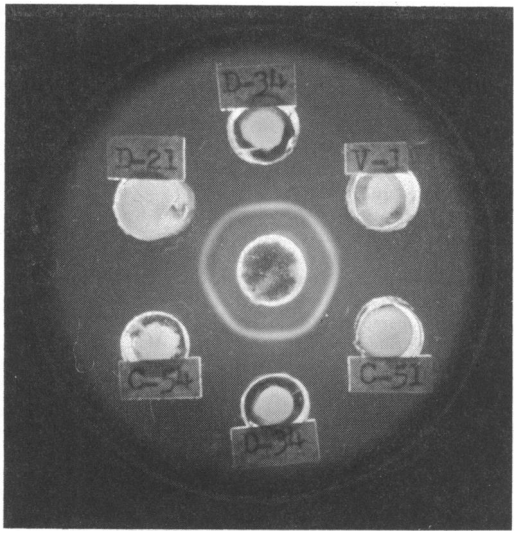

FIG. 4. *Agar-diffusion precipitin tests. Anti-D-34 (group II) serum in center well, group II cells in outer wells.*

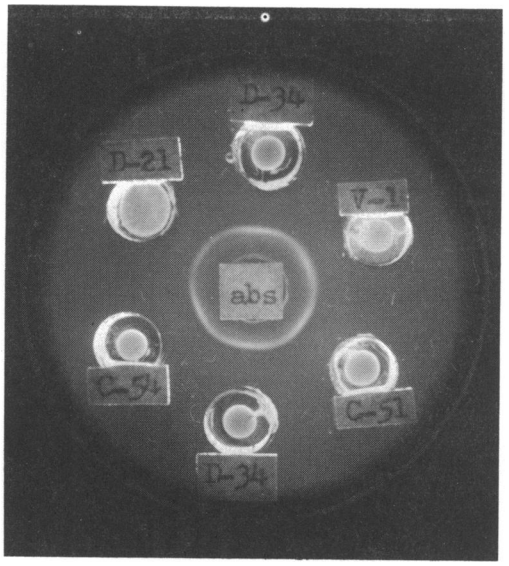

FIG. 5. *Agar-diffusion precipitin tests. Anti-D-34 (group II) serum absorbed with 6919 cells (group I) in center well, group II cells in outer wells.*

DISCUSSION

Descriptions of the anaerobic or microaerophilic species of *Corynebacterium* in Breed et al. (2) and Prévot (16) are not consistent, and are of limited value for taxonomic purposes. The suggestion of Zierdt et al. (22) that all anaerobic corynebacteria be classified as *C. acnes* perhaps goes too far [see Moore et al. (11)], but it is useful in emphasizing the close relationship or identity of many of the organisms which were described as separate species. Others (1, 5, 19) also stressed the close relationship or identity of *C. acnes* with *C. avidum*, *C. liquefaciens*, *C. parvum*, *C. granulosum*, and *C. anaerobium*. On the other hand, Werner and Mann (21) demonstrated differences in the sugars found in the cell walls of eight strains of *C. acnes* from those in one strain of *C. parvum*; the latter closely resembled the group II strains described here.

Of a much larger number of reactions which were tested, those cited in Table 2 were of greatest value in distinguishing between groups I and II. In light of the significance assigned to gelatin liquefaction in separating the two types, it should be noted that group II strains hydrolyze Kohn's charcoal gelatin pellets as used by Zierdt et al. (22); apparently, digestion of Formalin-denatured gelatin is not equivalent to digestion of untreated gelatin.

TABLE 3. *DNA base composition of strains of Corynebacterium acnes groups I and II*

Group	Strain	Per cent GC
I	6919	60.8
	6921	59.5
	11827	61.2
		60.5[a]
II	D-34	64.3
	C-51	63.3
	4-176	64.0
		63.9[a]

[a] Average.

Strains of both groups I and II were found to be generally lipolytic on tributyrin and triolein, in accord with the evidence of others (9, 18) that *C. acnes* produces lipase. There is, however, considerable variation in the lipolytic activity of various strains.

Although production of a variety of volatile fatty acids by the anaerobic corynebacteria was reported (20), representatives of both groups I and II were found in this study to produce only propionic acid. The two groups could not be thus distinguished, although there was a tendency for group I strains to produce more volatile acid than did group II.

Because Moss and Cherry (12) reported that *C. acnes* possesses the iso-C_{15} fatty acid as its principal fatty acid component, in contrast to the possession of a major anteiso-C_{15} fraction by two species of *Propionibacterium*, the fatty acids of three strains of each group were investigated and found to be indistinguishable. Both groups show a major peak corresponding to the iso-C_{15} acid, in agreement with the earlier report (12).

Other workers (1, 5, 19) showed marked antigenic relationships among the anaerobic diphtheroids, but were unable to distinguish satisfactorily between the closely related organisms by agglutination or hemagglutination tests. Use of agar diffusion precipitin tests in this work permitted a clear distinction between two closely related antigenic types, which conforms with differences in their biochemical activity.

Values of 48% GC for *C. acnes* DNA were reported (3, 10), although 58% GC was found in the very similar *C. parvum* (10). These values were determined by hydrolysis of the DNA and chromatographic separation of the bases, and are considerably below those found by Mandel. In view of the much higher value reported for *C. parvum*, there is reason to doubt the value of 48% GC assigned to *C. acnes*. Mandel's demonstration of a difference between DNA preparations isolated from representative strains of the two groups of *C. acnes* confirms the reality of the difference between groups I and II. Thus, differences in biochemical activity, phage susceptibility, antigenic specificity, and DNA composition all serve to distinguish between groups I and II.

On the basis of the evidence presented in this paper, those organisms comprising group I would seem to represent *Corynebacterium acnes*. The group II organisms fit neither the descriptions of *C. acnes* nor those of *C. avidum*, *C. parvum*, etc. (2, 16). Until more is known about the genetics of the two groups, it seems appropriate to retain the designation *C. acnes* group II for the latter organisms and to withhold application of a specific epithet, pending further clarification of the taxonomy of the anaerobic corynebacteria.

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