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Biotyping of *Propionibacterium acnes* Isolated from Normal Human Facial Skin

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Biochemical and serological characteristics of 128 strains of *Propionibacterium acnes* isolated from the facial skin of healthy Japanese volunteers were compared with the three standard strains of the American Type Culture Collection, ATCC 6919, 11827, and 11828. Accordingly, the isolated strains of *P. acnes* were classified into five biotypes (B1 to B5) on the basis of fermentation tests of ribose, erythritol, and sorbitol. Two serotypes were distinguished by the agglutination test. *P. acnes* belonging to serotype I had galactose as a cell wall sugar, whereas those of serotype II lacked galactose. The strains of serotype I were distributed among all five biotypes (B1 to B5); however, those of serotype II consisted only of one biotype (B2). A term "sero-biotype" was introduced to differentiate and carefully classify the isolates. The predominant sero-biotypes differed with the individual and region of the facial skin. In general, strains of sero-biotype IB1, IB3, IB4, and IIB2 were more frequently isolated than those of sero-biotype IB2 and IB5. Thus, for routine assay work, serotyping of *P. acnes* as based on erythritol and sorbitol fermentation is both practical and applicable.

Although an abundance of data on biochemical and serological studies of *Propionibacterium acnes* has been accumulated, there still remains a great deal of confusion regarding criteria for classifying the bacteria, and other species such as *P. granulosum* are often mistakenly included in the group (1, 9-14, 19).

Discrepancies related to taxonomy of *P. acnes* (3) were clarified by Johnson and Cummins (5). The bases that they used for classification of the bacterial species were (i) cell wall agglutination test, (ii) analysis of cell wall composition, (iii) determination of guanine + cytosine ratio, and (iv) identification by deoxyribonucleic acid-deoxyribonucleic acid homology. These criteria were subsequently supported by the editorial board of *Bergey's Manual* and appeared in the eighth edition (8).

In our previous communications, we discussed the determination of the optimal condition for isolation of *Propionibacterium* from normal human skin and the results of studies with biochemical tests to differentiate *P. acnes* and *P. granulosum* (7).

Described herein are the biochemical and serological characteristics of *P. acnes*, the study of which led to the development of a new method for the biotyping of bacterial species.

MATERIALS AND METHODS

Strains. One hundred twenty-eight strains of *P. acnes* isolated from facial skin of healthy Japanese volunteers were studied, together with the standard strains ATCC 6919, ATCC 11827 (serotype I), and ATCC 11828 (serotype II). The isolates were derived from the skin of the forehead, ala of the nose, cheek, and chin from 15 men and 15 women. The major characteristics and the methods of isolation and identification of *Propionibacterium* used in the present study were as described elsewhere (7).

Agglutination test for serotyping. (i) Preparation of antigens. The cells of *P. acnes* were cultured in 200 to 250 ml of broth medium (trypticase [BBL Microbiology Systems], 1.5 g/dl; yeast extract [BBL Microbiology Systems], 0.3 g/dl; heart extract (Nissui), 0.3 g/dl; glucose 1.0 g/dl; NaCl 0.2 g/dl; K_2HPO_4, 0.2 g/dl; L-cysteine HCl, 0.03 g/dl; Tween 80, 0.025 ml/dl [pH 6.8]) at 37°C for 3 to 4 days. The cells were then harvested, washed twice with distilled water, resuspended in a solution of thimerosal (20 mg/dl) at the density of McFarland nephelometer standard no. 5 and stored at 4°C. Immediately before use, the cells were homogenized by sonication for 5 to 10 s.

(ii) Preparation of antisera. The antisera were prepared by giving 2- to 2.5-kg rabbits three to five intravenous administrations of antigens of *P. acnes* ATCC 11827 and ATCC 11828. The dose was increased from 0.2 to 1.0 ml at intervals of 5 to 7 days. The antisera were collected 2 weeks after the final injection.

(iii) Absorption of antisera. The antisera were absorbed at a dilution of 10-fold with 0.1% NaCl solution with the pellets of antigens centrifuged at 5,000 rpm for 10 min from the density of McFarland nephelometer standard no. 8. The absorption mixtures were incubated at 37°C for 2 h under gentle shaking, after which the cells were removed by centrifugation.

(iv) Agglutination test. The tests were carried out in U-type well microtiter plates by incubating a mixture of antiserum and antigen in equal volumes of 0.025 ml. The plates were incubated at 37°C for 2 h and then overnight at 4°C before being read. The diluent for the antisera was 0.1% NaCl solution with 2% calf serum (16, 22).

Analysis of cell wall sugars. Analysis of cell wall sugars was by the method of Ushijima et al. (17). *P. acnes* cells were cultured in 200 to 250 ml of broth medium at 37°C for 3 to 4 days and then were suspended in 20 to 25 ml of distilled water and disrupted by a sonicator. The intact cells were removed by centrifugation at 2,000 × *g* for 20 min, and the cell wall fractions were collected by centrifugation at 10,000 × *g* for 30 min. The pellets were lyophilized. The lyophilized cell wall fractions (10 mg) were treated in sealed ampules with N/2 HCl-methanol complex at 100°C for 5 h. Free fatty acids were removed by extraction with petroleum ether. After evaporation to dryness, these samples were placed in a vacuum desiccator over silica gel and KOH pellets and allowed to stand overnight. The samples were then trimethylsilylated by the addition of 0.1 ml of trimethylsilylating reagent (hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine, Tokyo-Kasei) at a room temperature for 10 min and then analyzed by gas chromatography on a glass column (2 m by 3 mm) packed with 3% SE-52 on Chromosorb W (AW, DMCS, 80 to 100 mesh). The carrier gas was N_2 (40 ml/min); the oven temperature was 170 to 190°C, and the injection and detector temperature was 240°C. The identification of individual sugars was made by a comparison with the retention times of methanolyzed and trimethylsilylated galactose, glucose, and mannose.

Fermentation tests. Both basal medium (Trypticase [BBL Microbiology Systems], 1.0 g/dl; yeast extract [BBL Microbiology Systems], 0.3 g/dl; heart extract [Nissui], 0.3 g/dl; NaCl, 0.2 g/dl; L-cysteine-HCl, 0.03 g/dl; agar, 0.1 g/dl; Tween 80, 0.025 ml/dl [pH 7.0]) and carbohydrate medium (basal medium plus 1 g of carbohydrate per dl) were inoculated with Pasteur pipettes. Of the carbohydrates used, deoxyribose, ribose, and xylose were sterilized by filtration and added to autoclaved basal media. After incubation at 37°C for 7 days, the pH's of the cultures and uninoculated media were checked by pH meter. To obtain a more accurate value for the change of pH by the sugar per se, the "sugar-specific pH" (SpH) for each carbohydrate was calculated by the equation SpH = (A − B) − (C − D) (15, 18), where A is the pH of carbohydrate medium, B is the pH of culture of carbohydrate medium, C is the pH of basal medium, and D is the pH of culture of basal medium. SpH ≧ 0.35 was interpreted as fermentation positive, and SpH < 0.35 was interpreted as negative.

RESULTS

Serotyping. Two serotypes (I and II) as reported by Johnson and Cummins (5) were distinguished by the agglutination test (see Tables 1 and 3); i.e., 98 out of 128 strains reacted specifically with anti-*P. acnes* ATCC 11827 serum (serotype I) and the remaining 30 strains, with the anti-*P. acnes* ATCC 11828 serum (serotype II).

Cell wall sugars. There were two or three sugars detectable in the cell wall of *P. acnes*. All strains of serotype I were found to possess galactose, glucose, and mannose, but the strains of serotype II were devoid of galactose. *P. acnes* serotypes I and II were, therefore, clearly differentiated by presence or absence of galactose as a cell wall sugar (5).

Fermentation tests. All test strains were positive for galactose, glycerol, glucose, fructose, and mannose but negative for arabinose, cellobiose, glycogen, lactose, sucrose, maltose, starch, and xylose. However, the results with eight carbohydrates (adonitol, deoxyribose, erythritol, inositol, mannitol, ribose, sorbitol, and trehalose) were variable.

Biotyping. Ribose, erythritol, and sorbitol were fermented specifically by some strains, and in most cases the final pH of the cultures of these carbohydrate media reached less than 5.0 (SpH ≧ 1). Therefore, we chose these carbohydrates as determinant factors for biotyping of *P. acnes* (Table 1). As shown in Table 2, the results of fermentation tests led to the subdivision of all strains of *P. acnes* into five biotypes (B1 to B5). Standard strains of *P. acnes* ATCC 11827 belonged to B1, ATCC 11828 belonged to B2, and ATCC 6919 belonged to B3.

Relation between serotypes and biotypes. The serotype I strains were subdivided into five biotypes, and the serotype II strains were found to consist of only one biotype. We tentatively employed the term "sero-biotype" for expressing both serotype and biotype of one strain. Thus, the strains of serotype I were subdivided into five sero-biotypes, IB1 to IB5, and serotype II was into one sero-biotype, IIB2 (Table 3). The symbols, e.g., IB2 and IIB2, indicate the strains of serotype I biotype B2 and those of serotype II biotype B2, respectively. Standard strains of *P. acnes* ATCC 11827, ATCC 6919, and ATCC 11828 belonged to IB1, IB3, and IIB2, respectively.

Distribution of serotype and sero-biotype. With few exceptions, the strains of serotype I were predominant on facial skin. These predominant sero-biotypes differed with individual and also with the facial region. In general, sero-biotypes IB1, IB3, IB4, and IIB2 were isolated more frequently than were IB2 and IB5 (Table 4).

DISCUSSION

In 1972, Johnson and Cummins accurately classified *P. acnes* and interrelated propionibac-

TABLE 1. *Biochemical and serological tests of strains of P. acnes*

Strain	Galactose in cell wall	Agglutination test[a]		Acid from:		
		Anti-27	Anti-28	Ribose	Erythritol	Sorbitol
1[b]	+	80[c]	20	+	+	+
2	+	80	20	+	+	+
3	+	80	40	+	+	+
4	+	80	40	+	+	−
5	+	160	40	+	+	−
6	+	80	40	+	+	−
7	+	80	40	+	−	+
8	+	80	20	+	−	+
9	+	80	20	+	−	+
10	+	80	20	+	−	+
11	+	80	40	+	−	+
12	+	80	20	+	−	−
13	+	160	20	+	−	−
14	+	160	<20	+	−	−
15	+	80	40	+	−	−
16	+	80	20	+	−	−
17	+	40	<20	−	−	−
18	+	80	<20	−	−	−
19	+	40	<20	−	−	−
20	−	<20	80	+	+	−
21	−	20	160	+	+	−
22	−	<20	80	+	+	−
23	−	20	80	+	+	−
24	−	<20	160	+	+	−
6919[d]	+	80	40	+	−	+
11827	+	160	<20	+	+	+
11828	−	<20	80	+	+	−

[a] Absorbed anti-*P. acnes* ATCC 11827 and 11828 sera.
[b] Isolates from human skin.
[c] The highest final dilution at which complete agglutination occurred.
[d] ATCC strains.

teria and demonstrated that use of the cell wall agglutination test enables division of *P. acnes* into 2 serotypes. They also found that the strains of serotype I had galactose as a cell wall sugar constituent; however, such was not the case in serotype II (5).

Different groups of workers have attempted to subdivide *P. acnes* by serotyping (10, 19). Voss et al. reported their classification of the bacterial species on the basis of Brzin's report (1). Later they found that *P. granulosum* was included with the *P. acnes* (21) when they reexamined the characteristics of the strains used by the methods of Johnson and Cummins. Pulverer and Ko reported 11 serotypes of *P. acnes*, as defined by tube agglutination tests with five-factor antisera (10). However, four out of five strains that they used for preparation of antisera turned out to be *P. granulosum* (6), and later they reported that *P. acnes* consisted of only one serotype (U. Hoeffler, H. L. Ko, and G. Pulverer, personal communication).

In the present work, we arrived at the same conclusions as Johnson and Cummins, i.e., *P. acnes* can be divided into two serotypes by agglutination tests with two antisera prepared against ATCC 11827 (serotype I) and ATCC 11828 (serotype II). The presence or absence of galactose as a cell wall sugar also differentiated the two serotypes.

Three reports on biotyping of *P. acnes* have appeared in the literature (10, 13, 14). However, strains of *P. granulosum* were confused with *P. acnes*, as in the case of serotyping. In our work, the isolates of *P. acnes* were carefully identified following the guideline of the *Anaerobe Laboratory Manual* (4) and were subdivided into five biotypes by means of fermentation tests of three carbohydrates: ribose, erythritol, and sorbitol. We selected these particular carbohydrates as being sugars that fermented well with almost all strains of *P. acnes*.

Cummins and Johnson found that sorbitol fermentation was useful for the assay of serotypes of *P. acnes* (2). However, the substantial proportion of strains of serotype I were usually sorbitol fermentation negative (2, 8, 14). In our study also, only 54% of the strains of serotype I were sorbitol fermentation positive.

Serotypes of 94% strains of *P. acnes* could, however, be identified by combining the fermen-

TABLE 2. *Biotyping of P. acnes*

Biotype	No. of strains	Acid from:		
		Ribose	Erythritol	Sorbitol
B1 (11827)[a]	29	+	+	+
B2 (11828)	35	+	−	−
B3 (6919)	24	+	−	+
B4	21	+	−	−
B5	19	−	−	−

[a] Numbers in parentheses designate ATCC strains.

TABLE 3. *Relationships between biotype and serotype of P. acnes*

Serotype by agglutination	Sero-biotype	No. of strains	Galactose in cell wall	Acid from:		
				Ribose	Erythritol	Sorbitol
I	IB1 (11827)[a]	29	+	+	+	+
	IB2	5	+	+	+	−
	IB3 (6919)	24	+	+	−	+
	IB4	21	+	+	−	−
	IB5	19	+	−	−	−
II	IIB2 (11828)	30	−	+	+	−

[a] Numbers in parentheses designate ATCC strains.

TABLE 4. *Distribution of P. acnes on normal human skin*[a]

Region	Subject[b]	No. of *P. acnes* (cells/cm^2)	No. of strains isolated	Distribution (%) of sero-biotype					
				IB1	IB2	IB3	IB4	IB5	II B2
Forehead	1 (M)	7.3×10^6	10	80	—	—	10	—	10
	2 (F)	6.0×10^3	6	33	—	—	33	—	34
	3 (M)	1.4×10^6	5	40	—	—	60	—	—
	4 (F)	4.0×10^3	8	—	50	25	25	—	—
	5 (M)	6.3×10^5	10	30	—	—	—	—	70
	6 (F)	3.2×10^5	9	—	—	22	—	—	78
Cheek	1 (M)	2.0×10^5	13	85	—	—	—	—	15
	4 (F)	6.0×10^3	6	—	—	—	83	—	17
	2 (F)	4.0×10^3	4	25	—	—	75	—	—
	7 (F)	9.0×10^4	9	11	—	11	—	78	—
Ala of nose	1 (M)	6.0×10^6	6	—	—	100	—	—	—
	8 (M)	1.8×10^6	9	33	—	34	—	—	33
Chin	1 (M)	4.9×10^5	10	60	—	20	10	—	10

[a] The data summarized in this table were taken from 8 persons among 30 volunteers surveyed in the present study who yielded multiple strains of isolates.

[b] Letters in parentheses indicate the persons' sex.

TABLE 5. *Estimation of serotype by fermentation of erythritol and sorbitol*

Serotype	Biotype	No. of strains	Fermentation of:	
			Erythritol	Sorbitol
I	B3, 4, 5	64	−	
	B1	29	+	+
	B2	5	+	−
II	B2	30	+	−

tation data for sorbitol and erythritol. As shown in Table 5, all sorbitol-positive or erythritol-negative strains or both were serotype I and 86% of erythritol-positive strains among the sorbitol negatives were serotype II.

Webster and Cummins found that with bacteriophage typing serotype I and II of *P. acnes* could be distinguished (20). As a routine assay system, however, serotyping of *P. acnes* as based on erythritol and sorbitol fermentation is more practical and applicable.

We have also turned our attention to the distribution of sero-biotypes of *P. acnes* in acne vulgaris. In preliminary experiments, the predominant strain that we isolated from pustules was sero-biotype IB3. These findings will be reported elsewhere.

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