Isolation and Identification of *Peptococcus saccharolyticus* from Human Skin

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Peptococcus saccharolyticus was a numerically important organism on the forehead and the antecubital fossa of the arm of ca. 20% of the subjects studied. It was best detected by prolonged (4- to 7-day) anaerobic incubation on a suitably enriched medium but also appeared in smaller numbers in aerobic primary cultures after 4 to 7 days. Initial screening was based on growth patterns in shake cultures and on the relative amounts of growth on aerobic and anaerobic streak plates. The organism was nonhemolytic and usually catalase positive. It produced acid anaerobically from glucose, fructose, and glycerol, but not from maltose, and did not produce lactic acid from glucose.

In studies of the bacterial flora of normal human skin, *Peptococcus saccharolyticus* was one of the more important organisms we encountered. It was present on the forehead and arm (antecubital fossa) of ca. 20% of all subjects. When present, it was often more numerous than all other organisms combined.

In this paper we report characteristics of *P. saccharolyticus* that are helpful in its isolation and identification in cultures of human skin.

MATERIALS AND METHODS

Source of isolates. Subjects in this study were students, faculty, and employees of the University of Washington. Material was harvested from the skin of the forehead, arm, or back and quantitatively cultured by methods previously described (3). A wet cotton swab was used to collect bacteria from a circular area of skin, 3.2 or 4.5 cm in diameter. Serial dilutions in a dilute phosphate buffer with Triton X-100 (Rohm and Haas) (13) were cultured on our standard agar medium (TSY) containing 3% Trypticase soy (Baltimore Biological Laboratory), 1% yeast extract, and 0.5% Tween 80 (polysorbate 80, Atlas Chemical, Wilmington, Del.).

Bacterial strains studied. For many years, our routine procedure in studies of skin flora included quantitation of what appeared to be a homogeneous group of anaerobic gram-positive cocci. Because their proper classification was uncertain, we recorded these bacteria as Q (query) cocci. The criteria for identification as Q cocci were as follows: (i) In primary quantitative cultures, anaerobic gram-positive cocci with appropriate microscopic appearance and colonial morphology outnumbered all aerobic bacteria by a wide margin (at least threefold). (ii) No film was visible on the surface of agar shake cultures in TSY; usually there was a zone of suppressed growth in the upper part of the agar column. Staphylococci in a shake culture formed a heavy growth across the top of the agar as well as numerous colonies in the agar. (iii) The

area of confluent growth was heavier and the well-separated colonies were larger on the anaerobic streak plates than on parallel aerobic plates. (Confirmatory tests ii and iii were usually, but not always, carried out.)

Numerous isolates of Q cocci have been accumulated in frozen storage since 1972. From this collection we chose 25 isolates from nine male subjects, age 24 to 65, for special study. The site of origin was the forehead for 16 isolates, the scapula for 4 isolates, the deltoid for 3 isolates, and the antecubital fossa for two isolates. Of these, 19 were originally obtained from anaerobic primary cultures and 6 were from aerobic primary cultures. Two showed equal growth on aerobic and anaerobic TSY streak plates but definite zones of inhibition at the top of shake cultures. Two other isolates in shake cultures showed the usual absence of growth on the agar surface but no zone of inhibition at the top of the agar column. On streak plates, however, growth was better anaerobically than aerobically.

Tests of biochemical and cultural characteristics. Hemolysis was tested on 5% sheep blood agar with either BBL blood agar base or an enriched blood agar base (Pfizer brucella agar base). Plates were incubated aerobically and anaerobically. Sensitivity to 50 µg of lysostaphin per ml was determined by a tube test (7). Fermentation of glucose and mannitol was tested by the Baird-Parker method (12) or by the purple agar method (7) with screening tests; fructose, glucose, glycerol, and maltose fermentations were tested by the Virginia Polytechnic Institute (VPI) method (6) with species identification tests. Coagulase activity was determined by a conventional tube test (rabbit serum), and catalase tests were performed by emulsifying a colony in a drop of 3% H₂O₂.

Gas-liquid chromatography was performed on cultures in peptone-yeast broth containing 1% glucose after anaerobic incubation for 7 days. We used a Hewlett-Packard 5830A gas chromatograph with N₂ carrier gas, a flame ionization detection system, and a column with 15% SP-1220/1% H₃PO₄ on 100/120 Chromosorb W AW (Supelco, Inc., Bellefonte, Pa.).

RESULTS

Table 1 shows the biochemical and cultural characteristics of the 25 strains given special study and data from other strains that were subjected to variable, less-comprehensive testing. All strains gave highly uniform results that are fully compatible with the identification of *P. saccharolyticus*. Wesley Kloos was helpful in ruling out the genus *Staphylococcus* on the basis of a weak catalase response and a negative benzidine test in the four isolates he studied. Four of our isolates were extensively characterized and identified as *P. saccharolyticus* by the staff of the VPI Anaerobe Laboratory.

In primary cultures from skin incubated for 4

TABLE 1. *Reactions of P. saccharolyticus isolates*

Determination	No. of strains tested	Organisms with given reaction (%)
Catalase	180	
Positive		98
Negative		2
Coagulase	64	0
Growth on 5% sheep blood agar	25	
Anaerobically at 2 days		100
Aerobically at 2 days		0
Aerobically at 7 days		40
Hemolysis, 5% sheep blood		
Standard	25	0
Enriched	14	0
Hemolysis on bovine blood agar	4	0
Growth in shake culture	185	
No surface growth		100
Zone of growth inhibition		79
Growth on TSY at 4 to 7 days	178	
Only anaerobically		5
Better anaerobically than aerobically		76
Equally well aerobically and anaerobically		12
Slightly better aerobically		7
Carbohydrate fermentation		
Glucose	92	100
Glycerol	25	100
Fructose	25	100
Maltose	25	0
Lactic acid from glucose	25	0
Sensitivity to 50 µg of lysostaphin per ml	14	100

to 7 days, the number of *P. saccharolyticus* obtained anaerobically usually exceeded the number on aerobic cultures by a substantial margin (Table 2).

In exceptional cases, the number of colony-forming units in aerobic cultures came close to or even exceeded (Table 2, subject JP) the number in anaerobic cultures. However, *P. saccharolyticus* strains obtained originally from aerobic primary cultures were no different from those derived from anaerobic primary cultures. It was concern about this circumstance that caused us to include 6 isolates from aerobic primary cultures among the 25 given special study.

Cells of *P. saccharolyticus* were gram-positive, nonmotile, nonsporeforming cocci occurring in irregular masses or occasionally in tetrads or pairs. Colonies on TSY were raised, circular, opaque, and gray to white. In primary cultures from skin, colonies usually averaged less than 1 mm (range, 0.1 to 2.0 mm; median, 0.8 mm), in contrast to colonies of *S. epidermidis*, which were 3 to 6 mm in diameter.

All strains tested were coagulase negative. The catalase test was positive in 98% of isolates tested, although some gave a weak reaction compared with that of staphylococci.

In shake cultures, there was no visible film of growth of any strain on the agar surface exposed to air. Most isolates showed a zone of growth inhibition in the agar column ca. 3 mm wide in standard medium and 10 mm wide in glucose-free medium. A band of maximal growth ca. 2 mm wide was usually present immediately below the zone of inhibition. On duplicate streak plates of TSY incubated aerobically and anaerobically, 76% of the isolates grew better anaerobically than aerobically. Half of the others appeared to grow equally well aerobically and anaerobically, and a small number grew only anaerobically.

TABLE 2. *Colony-forming units of P. saccharolyticus on duplicate aerobic and anaerobic primary skin cultures^a*

Sub- ject	Site ^b	CFU/cm ² of skin ^c		
		Aerobic	Anaerobic	Ratio
JF	Forehead, 1	7,750	70,000	1:9
JF	Forehead, 2	8,250	57,500	1:7
JF	Forehead, 3	1,620	57,500	1:35
WG	Forehead, 1	1,050	7,000	1:6.6
WG	Forehead, 2	2,700	6,500	1:2.4
JP	Forehead	1,280	1,000	1:0.8
CE	Deltoid	210	740	1:3.5
CE	Antecubital			
	Fossa	3,160	10,320	1:3.3

^a Incubated 4 to 7 days.

^b Tests 1, 2, and 3 were of the same site at different times.

^c CFU, colony-forming units.

The few that showed more growth aerobically than anaerobically showed a clear zone of inhibition at the top of each shake culture.

On blood agar at 2 days, growth was visible on anaerobic but not on aerobic cultures; at 7 days, the areas of heavy inoculation showed scant growth aerobically on some cultures. No hemolysis was observed.

On Baird-Parker medium, acid was produced anaerobically from glucose but not from mannitol. Details of other carbohydrate tests are included in Table 1.

DISCUSSION

Classification of *P. saccharolyticus* from the forehead. Peptococci are, by definition, anaerobes. Although most peptococci are more rigorous in their requirements for anaerobiosis, our isolates are still within the definition of anaerobes as "organisms that thrive best, or only, when deprived of oxygen" (1). The classification of our organism is based on its description (i) by Foubert and Douglas (5), (ii) in the 7th edition of *Bergey's Manual of Determinative Bacteriology* (2), and (iii) in the *VPI Anaerobe Laboratory Manual* (6). Schliefer and Nimmerman (11) concluded that *P. saccharolyticus* is not a peptococcus on the basis of the peptidoglycan structure of its cell wall. The species *P. saccharolyticus* has been excluded from the genus *Peptococcus* in the 8th edition of *Bergey's Manual* (10) on the basis of its fermentative capabilities. However, the validity of this organism as a bona fide species has not been questioned. It is unfortunate that it was omitted from *Bergey's Manual* because an acceptable generic taxonomic assignment for it was not established.

A relationship between *P. saccharolyticus* and *S. epidermidis* is suggested by a similarity in the structure of their peptidoglycans (11). The fact that lactic acid is not a product of fermentation of glucose by *P. saccharolyticus*, however, establishes a considerable difference between the two. The failure to produce lactic acid also clearly separates *P. saccharolyticus* from the genus *Streptococcus* and probably from *Aerococcus*. (*A. viridans* ATCC 11563 produced lactic acid in our tests.)

In their description of *P. saccharolyticus*, Foubert and Douglas (5) state that all of the strains they studied "are strictly anaerobic and have shown no tendency to become facultatively anaerobic." Foubert reports these studies in greater detail, giving as evidence for this statement the consistent presence of a zone of growth inhibition at the top of the agar column in shake cultures (Ph.D. thesis, University of Washing-

ton, Seattle, 1947). Although our observations were generally consistent with theirs, we found that most isolates of *P. saccharolyticus* are more tolerant of oxygen than Foubert and Douglas realized.

Significance of *P. saccharolyticus* in the indigenous flora of skin. Of the 40 subjects whose foreheads we studied, 8 (20%) were carrying *P. saccharolyticus*. Sixteen of these persons were cultured four to nine times each over periods of 12 to 54 months. Three subjects yielded *P. saccharolyticus* in every test. On two subjects, it outnumbered all other organisms in every culture.

Six (ca. 24%) of the 25 subjects cultured harbored *P. saccharolyticus* in their antecubital fossa. On two subjects it was the predominant organism. One of these subjects was cultured four times over a period of 7 months, and in each test *P. saccharolyticus* was predominant.

In past studies of the flora of normal human skin, *P. saccharolyticus* was not recognized as a significant organism. In the two comprehensive reviews of cutaneous flora published in recent years (8, 9) it is not mentioned in the discussion of normal (or abnormal) flora.

That this organism was not recognized in past studies is puzzling in view of its prevalence and the fact that its original description was based on 13 isolates, 8 of which came from the skin of the upper arm of three subjects (E. L. Foubert, Jr., personal communication). Evans et al. (4) also noted its presence as early as 1950 but provided little information about its numbers or frequency of occurrence.

The following factors may have militated against the detection of *P. saccharolyticus* in other studies: (i) the culture media were not satisfactory (we found the Marshall-Kelsey medium, which has been used in many studies of skin flora, inadequate for this organism), (ii) incubation was not long enough, (iii) anaerobic cultures were not employed, or (iv) the organism was encountered but not correctly identified.

From our experience, it appears the effective measures to increase the detection of *P. saccharolyticus* in cultures of human skin or in clinical specimens would include: (i) extending the incubation time beyond the usual 1 or 2 days, (ii) making anaerobic subcultures of appropriately selected colonies that appear on aerobic primary cultures, and (iii) doing a catalase test on isolates of anaerobic gram-positive cocci that do not form definite long chains of cells.

For practical purposes, the identification is confirmed if the organism grows best anaerobically, is nonhemolytic, and produces acid from glucose, fructose, and glycerol but not from maltose.

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