

Regional Variations of Cutaneous Propionibacteria

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Propionibacterium acnes, *P. avidum*, and *P. granulosum* were quantitatively measured in 50 young adults. The scalp, forehead, external auditory canal, alae nasi, anterior nares, groin, rectum, and antecubital and popliteal fossa were sampled. These represent various cutaneous microenvironments, differing in moisture, density of sweat, sebaceous glands, and extent of anaerobiosis. These studies show that the propionibacteria are ubiquitous on the skin, with *P. acnes* predominant in both prevalence and population, especially in areas rich in sebum. *P. granulosum* recovery paralleled that of *P. acnes*, but the density was significantly lower. *P. avidum* was found mainly in moist areas and the rectum, suggesting an intestinal reservoir.

The only anaerobic organisms shown to be established and proliferating members of the cutaneous microflora are the propionibacteria (13). Recent investigations have led to more precise methods for the identification of this genus as to species, and three main species have been identified on human skin, namely, *Propionibacterium acnes*, *P. granulosum*, and *P. avidum* (2, 3, 14). Previous workers have reported quantitative differences for various body regions, but their studies, for the most part, were conducted before the development of accepted methodology for identification as to species (5, 13, 15). For example, Evans et al. (5) in 1950 showed that the ear lobe supported in the range of 10^5 propionibacteria per cm^2 compared with 10^4 for the shoulder and deltoid and 10^1 for the palm. Somerville and Murphy (19) sampled multiple areas and found *P. acnes* in all 22 subjects, ranging from $10^4/\text{cm}^2$ for the forehead, chest, and upper neck to 10^1 in the extremities. Neither study classified propionibacteria by species, although, in retrospect, the criteria of Evans et al. for identification as to species could have eliminated *P. granulosum* strains. Marples and McGinley (14) sampled five areas quantitatively and found *P. acnes* to be the most prevalent as well as the highest in total number of any other species. *P. granulosum* was the next most prevalent and numerous species. *P. granulosum* was the most prevalent and numerous species, with *P. avidum* infrequent except in the axilla, where it was present in 24 of 46 sites with an average density of $10^4/\text{cm}^2$. With the exception of the axilla, all four other sites were sebaceous-rich areas, and these findings may have represented too limited a sampling of the various cutaneous ecologies. Moreover, that study was not con-

trolled with respect to age and sex. We have recently demonstrated that at least on the forehead there are profound age-related changes, with *P. acnes* levels being quite low until the midteens or early twenties and increasing steadily into the thirties and forties (10). Differences in *P. acnes* levels between the sexes did not occur in children and teenagers, but beyond age 25, males clearly carried greater numbers of *P. acnes*. Our interpretation of these findings was that *P. acnes* levels paralleled age- and sex-related differences in sebaceous gland activity (4, 17).

Interest in this genus has centered mainly around its role in the pathogenesis of acne vulgaris. It has been established that acne patients support several orders of magnitude more *P. acnes* in the acne area than age-matched controls and that suppression of this organism is accompanied by clinical improvement (8). Renewed interest in this genus has been sparked by the work of Cummings and Johnson, who showed that most *Corynebacterium parvum* strains are in reality *P. acnes* (3). Because *C. parvum* has been shown to have a profound stimulating effect on the immune system and can enhance suppression of tumors in animals and humans (7), the possibility exists that human propionibacteria can serve an immunostimulatory role. It has even been proposed that acne vulgaris may serve as a biological protection against the development of cancer (18).

In summary, previous investigations have indicated that profound differences occur with respect to both the total number and the kind of propionibacteria found in various body regions. In light of the potential significance of this genus, we undertook to define more pre-

cisely the prevalence and density of cutaneous propionibacteria in a defined, homogeneous group to search for qualitative and quantitative differences that might provide insights into the ecology of this genus on human skin.

MATERIALS AND METHODS

Subjects. Care was taken to assure that the subject group was as homogeneous as possible and that they exhibited no acne, seborrhea, psoriasis, or any other skin disorder, because we have shown previously that propionibacteria levels are significantly lowered in dermatitic skin (9, 12).

Fifty healthy, adult males between the ages of 18 and 28, with a mean age of 26, were studied. We limited this survey to males to eliminate any possible sex differences or hormonal influences such as the menses or use of birth control pills. These restrictions were deemed important because sites rich in sebaceous glands (scalp, ear, forehead, and alae nasi) can have significant variation in sebum production as a result of hormonal stimulation. Moreover, we have already detected that in subjects over age 20 there is a significant sex-related difference in the total number of propionibacteria that parallels sex-related differences in sebaceous gland activity (4, 10, 17).

Sites sampled. We selected areas that differed in the amount of water and sebum available to bacteria. The oily areas were the scalp, forehead, external auditory canal, and the expressed contents of follicles in the alae nasi. The dry areas were the antecubital and popliteal fossae, whereas the wet areas were the anterior nares, axillae, groin, and rectum.

Culture technique. The scalp, forehead, arm, leg, axilla, and groin were sampled by the detergent scrub technique of Williamson and Kligman (22). This method involves placing a sterile glass cylinder with an internal area of 3.8 cm² over the area to be sampled, adding 1 ml of 0.1% Triton X-100 in 0.075 M phosphate buffer (pH 7.9), scrubbing with a blunted Teflon spatula for 1 min, and then withdrawing the sample fluid. This procedure is repeated, and the samples are pooled. The expressed contents of follicles in the alae nasi were weighed and then homogenized in 2 ml of 0.1% Triton X-100. The ear, anterior nares, and rectum were sampled by rubbing the area 10 times with a calcium alginate swab moistened in 0.1% Triton X-100. The swab was then placed in a tube containing 2 ml of 0.1% Triton X-100 and blended vigorously in a Vortex mixer. This method has been shown by Evans and Stevens (6) to yield reproducible quantitative results. Samples were processed by making 10-fold dilutions in half-strength scrub fluid (0.05% Triton X-100) to maintain the dispersion of bacteria. A 0.025-ml volume of each dilution was placed on brain heart infusion agar (BHIA; BBL), supplemented with 0.8% dextrose, 0.1% Tween 80, 0.5% yeast extract, 1% sodium lactate, and 0.5% of a salt solution containing 4 g of MgSO₄ · 7H₂O–0.4 g of MnSO₄ · 4H₂O–0.4 g of FeSO₄ · 7H₂O acidified with 2 drops of 10 N H₂SO₄ (BHIA+). Plates were incubated for 7 days at 37°C in a GasPak anaerobic system (BBL). After incubation, the numbers of colonies of the various species were counted. Scrub samples were then calculated to per square centimeter of skin surface, swabs being

expressed as per 2-ml sample and squeezings as per 0.1 mg of wet weight.

Marples and McGinley (14) have previously shown that the cutaneous propionibacteria can be selected on the basis of colonial morphology on primary subcultivation.

In this study, at least two distinctive colonial types from each site were initially subjected to susceptibility to *P. acnes* bacteriophage and the following biochemical tests. After 408 strains were studied (Table 1), the correlation between colonial morphology on primary subcultivation, susceptibility to *P. acnes* bacteriophage, and clearing of litmus milk agar (LMA) was found to be sufficient for identification as to species. Subsequently, all morphological types were subjected to phage susceptibility and LMA tests.

Phage susceptibility. *P. acnes* bacteriophage (ATCC 29399B) was titrated on *P. acnes* (ATCC 6919) lawn plates in 10-fold dilutions. The lowest dilution of phage producing confluent lysis was then applied to the unknown isolates on BHIA+ and incubated for 2 days.

Biochemical tests. A heavy suspension of each organism in 1% peptone water was prepared from a fresh culture. Broth media were seeded with 4 drops of this suspension. Solid media were inoculated by lightly pressing a cotton-tipped swab saturated with the broth suspension on the surface of the medium. This produced a uniform circular area of growth 3 to 4 mm in diameter. All tests were incubated for 7 days at 37°C with plated media in GasPak jars.

The production of catalase was assayed by applying 1 drop of 3% H₂O₂ to colonies on a BHIA+ plate that had been exposed to oxygen for 1 h. Indole production and nitrate reduction were tested in indole-nitrate broth (BBL), and gelatinase activity was tested in Thiogel medium (BBL). The ability to hydrolyze casein was assayed on LMA prepared by the method of Webster and McGinley (21). Zones of clearing were measured and correlated with known values for each species.

RESULTS

Table 1 summarizes the biochemical reactions for 408 propionibacteria. These colonies of an-

TABLE 1. *Biochemical classification of 408 cutaneous propionibacteria*

Test	Classification of propionibacteria					
	<i>P. acnes</i>		<i>P. granulosum</i>		<i>P. avidum</i>	
	+	-	+	-	+	-
Catalase	231	0	125	0	52	0
Phage ^a	229	2	0	125	0	52
Indole	201	30	0	125	0	52
Nitrate	230	1	0	125	0	52
Gelatin	231	0	0	125	52	0
LMA ^b	231	0	0	125	52	0

^a ATCC 29399B, phage A of Marples and McGinley.

^b Webster and McGinley (21). Zone sizes for *P. acnes* were <1.3 cm and those for *P. avidum* were >2.2 cm.

aerobic, gram-positive diphtheroids had representative colonial morphologies and were further identified by biochemical testing as a check on the accuracy of visual identification. Strains were classified as *P. acnes* if they were positive for catalase, gelatin liquefaction, and indole and/or nitrate reduction, were susceptible to *P. acnes* phage, and produced a clearing zone of no larger than 1.5 cm on LMA. *P. granulosum* strains were catalase positive, produced no indole, did not reduce nitrate, did not liquefy gelatin, did not clear LMA, and were resistant to *P. acnes* phage lysis. *P. avidum* was catalase and gelatinase positive, produced a zone of more than 2 cm on LMA, was not lysed by phage, and produced no indole or nitrate reductase.

Biochemical tests on 408 strains were performed as a check on the accuracy of identification by colonial morphology; 100% agreement was observed. Subsequently, all gram-positive anaerobic diphtheroids were identified by colonial morphology, *P. acnes* bacteriophage susceptibility, and LMA reactions.

Propionibacteria were present in practically every sample from oily areas (92 to 100% recovery from the external auditory canal, scalp, forehead, and alae nasi) and almost as frequently in wet areas (76 to 88% incidence), whereas the drier arm and leg areas supported propionibacteria less often (38 to 62%). By far the most prevalent species was *P. acnes*, and its density far exceeded the other species. Regional variations in species recovery and total population were also evident. *P. acnes* was present in all sites (38 to 100%), whereas *P. granulosum* was

frequently recovered from the wet and oily areas, particularly in the material expressed from the alae nasi, but was relatively sparse in the extremities. *P. avidum* was found only in the wet areas (axilla and groin) as well as in the anterior nares, alae nasi, and rectum (Table 2 and Fig. 1).

DISCUSSION

It has been stated frequently that the propionibacteria are among the most numerous organisms residing on human skin (5, 14, 19). No one, with the exception of Marples and Mc-

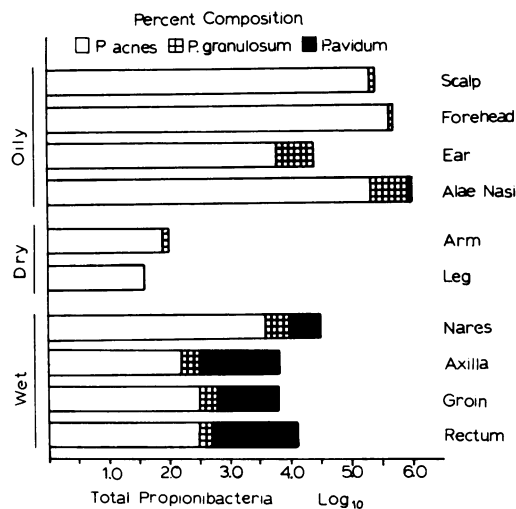


FIG. 1. Regional variation in composition and density of propionibacteria.

TABLE 2. Incidence and density of propionibacteria from 50 subjects

Site	Total propionibacteria			<i>P. acnes</i>			<i>P. granulosum</i>			<i>P. avidum</i>		
	%I ^a	Density ^b	SEM ^c	%I	Density	SEM	%I	Density	SEM	%I	Density	SEM
Oily												
Scalp	100	2.3×10^5	0.15	100	2.2×10^5	0.15	29	1.8×10^4	0.21	0		
Forehead	100	5.2×10^5	0.13	100	5.1×10^5	0.13	26	1.2×10^4	0.20	0		
Ear	92	2.4×10^4	0.11	79	4.1×10^4	0.16	32	1.5×10^3	0.26	0		
Alae nasi	100	1.1×10^6	0.11	100	9.1×10^5	0.11	85	7.3×10^4	0.13	9	1.4×10^4	0.37
Dry												
Arm	62	1.0×10^2	0.18	56	1.9×10^2	0.17	12	8.0×10^2	0.48	0		
Leg	38	4.3×10^1	0.16	38	4.3×10^1	0.16	0			0		
Wet												
Nares	88	2.9×10^4	0.14	79	2.8×10^4	0.12	18	2.3×10^3	0.42	26	1.2×10^4	0.23
Axilla	79	6.0×10^3	0.25	47	6.5×10^3	0.36	6	4.7×10^3	0.35	32	6.7×10^3	0.33
Groin	79	6.4×10^3	0.24	62	7.9×10^3	0.28	9	4.5×10^3	0.17	44	2.6×10^3	0.21
Rectum	76	1.4×10^4	0.21	56	1.4×10^4	0.25	6	6.0×10^2	0.15	41	2.3×10^4	0.18

^a %I, Percent incidence from 50 sites.

^b Geometric mean counts of propionibacteria when present expressed as (i) per square centimeter (scalp, forehead, arm, leg, axilla, groin); (ii) per 2-ml swab (ear, nares, rectum); (iii) per 0.1 mg of wet weight (alae nasi squeezings).

^c SEM, Standard error of the mean.

GINLEY (14), has studied the relative numbers or frequency of occurrence of propionibacterial species. In this study we have documented a high frequency of occurrence for propionibacteria as well as the relatively high numbers of these organisms found in some body areas. We studied a defined population of males, ages 18 to 28, to avoid age- and sex-related differences, both of which we have observed previously during our studies on acne (10). Our aim was to investigate the effect of different cutaneous environments on the incidence and density of the propionibacteria. Such regional qualitative and quantitative differences have been firmly established for the aerobic cutaneous microflora (1, 9-13, 16, 20). Many factors influence the composition and density of this population, the most potent of which are the extent of hydration and substrate availability (13). For example, areas rich in sweat glands support a dense population of aerobic cocci and lipophilic and large-colony diphtheroids, as well as significant populations of gram-negative rods (1, 9, 11, 13, 16, 20). Thus, in areas such as the axilla, groin, and toe web space, the average density of aerobes was in the range of 10^6 organisms per cm^2 , with a significant proportion of gram-negative species. In contrast, relatively dry areas such as the forearm and leg support far fewer organisms (10^2 to $10^3/\text{cm}^2$), which are mainly gram-positive cocci with low levels of diphtheroids and only a transient incidence of gram-negative species (9, 13, 16). Such regional variations support the concept that water is important for the establishment of a thriving gram-negative population. This is further supported by studies showing that occlusion (and the ensuing hydration) of normally dry areas results in an elevation of the number of bacteria present and a dramatic increase in the species proportion of gram-negative and diphtheroidal species (9).

Our results indicate that *P. acnes* is the most prevalent species and usually the most numerous of the three cutaneous propionibacteria on human skin. Certainly in the oily areas, such as the scalp, forehead, ear, and alae nasi, *P. acnes* was far more commonly found than *P. granulosum*, and the average density was approximately one order of magnitude greater. *P. acnes* was also very frequently found in areas rich in eccrine sweat (axilla and groin) as well as in mucosal areas such as the anterior nares and the rectum. The total density, however, was significantly lower for all, suggesting that sebum is an important substrate for *P. acnes*. *P. granulosum* showed a pattern similar to that of *P. acnes*; namely, the highest numbers and greatest frequency of recovery were in the sebum-rich areas supporting fewer organisms. The high in-

cidence of *P. granulosum* in the alae nasi suggests that this may be the headquarters of this species. For both species, it appears that sebum and water are important environmental determinants. *P. avidum*, on the other hand, was recovered only from the axilla, rectum, and anterior nares. These areas are moist rather than oily and are also populated with gram-negative organisms of enteric origin (1, 9, 13, 16, 20), possibly suggesting intestinal and/or respiratory reservoirs for this species. The recovery of *P. avidum* from the material expressed from the alae nasi may represent a spread from the anterior nares, just as the recovery in the groin suggests spreading from the rectum. The failure to recover this species from dry areas and, in contrast to other species, the lack of recovery in oily areas suggest that water is the dominant environmental force for *P. avidum*, with the anterior nares and possibly the gastrointestinal tract serving as headquarters.

These differences may be significant in their effect on human immunoregulatory functions. If *P. acnes* is truly important in immunoregulation, one would expect that it would be abundant in areas such as the anterior nares and gastrointestinal tract because these are areas of low barrier function, where any immunostimulatory material could exert its effect more readily. In contrast, intact skin possesses a formidable barrier with stratified keratinized cells on the surface and the lining of follicles, limiting the movement of external and internal substances. *P. acnes* and *P. granulosum* are relatively numerous in the more permeable mucosal sites (ranging up to 10^4 organisms per swabbing) and could thus stimulate the immune system of the host. Of interest and potential importance is the relatively high incidence and density of *P. avidum* in these areas. To our knowledge, this species has not been tested in the systems in which *P. acnes* strains have been shown to exert profound immunostimulatory effects. Its abundance in areas where it could exert systemic effects on the host indicates a need for further study.

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