Quantitation of Major Human Cutaneous Bacterial and Fungal Populations[▽]

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Because the human skin microbiota may play roles in the causation or modification of skin diseases, we sought to provide initial quantitative analysis from different cutaneous locations. We developed quantitative PCRs to enumerate the total bacterial and fungal populations, as well as the most common bacterial and fungal genera present in six locales, in eight healthy subjects. We used a set of primers and TaqMan MGB probes based on the bacterial 16S rRNA and fungal internally transcribed spacer region, as well as bacterial genus-specific probes for *Propionibacterium*, *Corynebacterium*, *Streptococcus*, and *Staphylococcus* and a fungal genus-specific probe for *Malassezia*. The extent of human DNA contamination of the specimen was determined by quantitating the human housekeeping *GAPDH* gene. The highest level of 16S rRNA copies of bacteria was present in the axilla (4.44 ± 0.18 $\log_{10}$ copies/μl [mean $\pm$ standard error of the mean]), with normalization based on *GAPDH* levels, but the other five locations were similar to one another (range, 2.48 to 2.89 $\log_{10}$ copies/μl). There was strong symmetry between the left and right sides. The four bacterial genera accounted for 31% to 59% of total bacteria, with the highest percent composition in the axilla and the lowest in the forearm. *Streptococcus* was the most common genus present on the forehead and behind the ear. *Corynebacterium* spp. were predominant in the axilla. Fungal levels were 1 to 2 $\log_{10}$ lower than for bacteria, with *Malassezia* spp. accounting for the majority of fungal gene copies. These results provide the first quantitation of the site and host specificities of major bacterial and fungal populations in human skin and present simple methods for their assessment in studies of disease.

Human skin harbors a diverse group of microorganisms that form complex communities and occupy specific niches and microenvironments (5, 9, 12, 14, 15). While most organisms colonizing the human body may be beneficial for health (4, 9, 18), some relationships with the host can change from commensal to pathogenic for reasons that are poorly understood (1). The global composition and presence of specific organisms also is relevant to the biological effects of the skin microbiota. The human cutaneous surface includes regions with diverse pH, temperature, moisture, and sebum content (14, 17), and skin structures such as hair follicles, sebaceous, eccrine, and apocrine glands comprise subhabitats that may be associated with their own unique microbiota (20).

Until recently, our knowledge of the bacterial biota in human skin has been based mostly on cultivation studies, which are insufficient because many organisms cannot be cultured (6). The direct PCR amplification and sequencing of bacterial genes encoding the small subunit rRNA (16S rRNA) or variable region fragments thereof has been a powerful method to analyze the enormous variation in the human microbiome (8, 24, 30), showing important differences among a number of cutaneous sites (5, 9, 12, 15). However, analysis of ribosomal genes (16S rRNA or intergenomic 18S and 23S rRNA) by using clone libraries or high-throughput sequencing may not allow for an accurate enumeration due to a variety of artifacts (19, 31). However, quantitative real-time PCR (qPCR) may be used as an alternative methodology (7, 23).

Based on our prior studies, which characterized the human forearm cutaneous microbiota (12, 13, 25, 26), we sought to quantify the human cutaneous microbiota from several body locations. The aim of the present study was to develop qPCRs to enumerate total bacterial and fungal populations, as well as to determine the most common bacterial and fungal genera in different locations on human skin.

MATERIALS AND METHODS

Subjects. Specimens from skin were obtained from eight healthy adult subjects (four males and four females) from 11 body locations, including the forehead, left and right axillae, left and right inner elbows, left and right forearms, left and right forelegs, and behind the left and right ears. To maximize the continuity of our investigations, two of the eight subjects (subjects 1 and 3) had been examined in a prior study (12), but new samples were obtained for this study. From two of the subjects (subjects 1 and 2), a second set of samples was collected 1 month later at the same time of day (afternoon). The subjects were not provided with any specific instructions before the samples were taken. The mean age of the subjects was 38 years (range, 25 to 58); all were in good health and had not received antibiotics for at least 4 months. The study was approved by the NYU Institutional Review Board, and all subjects provided written informed consent.

Specimen processing. Methods for specimen processing have been described elsewhere (12). In brief, a 2- by 2-cm area of the cutaneous surface at each of the 11 locations was sampled by swabbing the skin with a cotton pledget that had been soaked in sterile 0.15 M NaCl with 0.1% Tween 20 (Fisher Scientific, Fair Lawn, NJ). DNA was extracted from the swab suspensions in a PCR-free clean room by using the DNeasy blood and tissue kit (Qiagen, Chatsworth, CA); glass beads (0.5 to 1 mm) were added to the specimens and vortex mixed at maximum speed for 40 s, followed by DNA extraction, using the manufacturer's protocol

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TABLE 1. Primers and probes used in this study

Designation	Primer or probe	Target	Sequence (5′→3′)
8F	Primer	Bacteria	AGAGTTTGATYMTGGCTCAG
Eub361R	Primer	Bacteria	CGYCCATTGBGBAADATTCC
Eub519F	Primer	Bacteria	CAGCAGCCGCGGTRATA
U785R	Primer	Bacteria	GGACTACCVGGGTATCTAAKCC
ITS1F	Primer	Fungi	CTYGGTCATTTAGAGGAAGTAA
ITS2	Primer	Fungi	RCTGCGTTCTTCATCGWTG
Mal1F	Primer	Genus *Malassezia*	TCTTTGAACGCACCTTGC
Mal1R	Primer	Genus *Malassezia*	AHAGCAAATGACGTATCATG
GAPF	Primer	Human *GAPDH*	GGGCTCTCCAGAACATCATCC
GAPR	Primer	Human *GAPDH*	GTCCACCACTGACACGTTGG
Bacteria_P	Probe	Bacteria	TACGGGAGGCAGCAGT
Strep_P	Probe	Genus *Streptococcus*	AGATGGACCTGCGTTGT
Staph_P	Probe	Genus *Staphylococcus*	CTGTAACTGACGCTGATGTG
Prop_P	Probe	Genus *Propionibacterium*	CTTTCGATACGGGTTGACTT
Coryne_P	Probe	Genus *Corynebacterium*	ACAGYACTCHAGTHATGCCCGT
Fungal_P	Probe	Fungi	TCYGTAGGTGAACCTGCRG
Mala_P	Probe	Genus *Malassezia*	ATGCCTGTTTGWGTGC
GAP_P	Probe	Human *GAPDH*	CCTCTACTGGCGCTGCCAAGGCT

for genomic DNA isolation from Gram-positive bacteria, and samples were eluted in 100 μl AE buffer (DNeasy Blood and Tissue kit; Qiagen). To eliminate bacterial or DNA contamination, lysozyme (Sigma-Aldrich, St. Louis, MO) was passed through a microcentrifuge filter (molecular mass threshold, 30,000 Da; Amicon, Bedford, MA) at $18,514 \times g$ in a centrifuge (Eppendorf, Germany) for 20 min before adding to the enzymatic lysis buffer.

Quantitative PCR. We used sets of primers and TaqMan MGB probes (Table 1), based on the bacterial 16S rRNA sequences (12), the fungal internally transcribed spacer (ITS) sequences (25; http://pmb.berkeley.edu/~bruns/tour /primers.html), and the human glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) (28). In addition, we developed genus-specific approaches for *Propionibacterium*, *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Malesezzia*, because these have been the most common genera identified in studies using universal primers (5, 9, 12, 15, 25, 26). PCRs were performed using 3.5 mM MgCl₂, 0.4 ng/μl bovine serum albumin, 0.2 mM each deoxynucleoside triphosphate, 10 pmol of each primer, 5 pmol of each probe, 0.625 U *Taq* DNA polymerase (Qiagen), and 2 μl extracted DNA in a final 25-μl volume. PCR conditions were 5 min at 94°C and 45 cycles of 10 s at 94°C, 45 s at 54°C (for primers 8F/Eub361R) or 56°C (for primers Eub519R/U785R), and 60 s at 72°C. The assays were performed using a Rotor-Gene 6000 system (Corbett Life Science, Sydney, Australia), and a standard curve was constructed by using serial dilutions of cloned PCR products corresponding to each tested species. Each sample was tested at least twice, and the results were analyzed using the Rotor-Gene 3000 v.6.1.81 software.

Statistical analysis. Statistical analysis was performed with the Paleontological Statistics Software Package for Education and Data Analysis (http://palaeo -electronica.org/2001_1/past/issue1_01.htm). All qPCR results are presented as the means of the log₁₀ value ± the standard errors of the means (SEM). Statistically significant differences were determined by *t* tests and one-way analysis of variance (ANOVA) tests for independent samples. ANOVA tests also were conducted using SAS 9.2 statistical package for Windows (SAS Institute Inc., Cary, NC) with the PROC GLM procedure. Total variations accounted for by the model and by site, subject, side, and time were based on estimates for the omega-square and semipartial omega-square values. Statistical significance was defined as a *P* value of <0.05. The linear regression method was used to assess the correlation between the proportions of bacteria and fungi from samples obtained from the subjects' left and right sides.

RESULTS

Validation of TaqMan MGB probes. To examine the specificity of the probes, all probes first were validated through the Probe Match algorithm of the Ribosomal Database Project (RDP; http://www.rdp.cme.msu.edu/probematch). Subsequently, 16S rRNA gene PCR products from the most common skin bacteria genera (12) were used to evaluate the specificity of the probes. The products studied included members of the genus *Propionibacterium* as well as *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Rothia*, *Gemella*, *Micrococcus*, and *Kocuria* species (12). In each case, as expected, the genus-specific probes recognized the cloned DNA from the species within the same genus but not the other common skin genera (data not shown). Assessment of sensitivity was performed using 10-fold dilutions of the DNA templates used for the standard curves, corresponding to 3×10^1 to 3×10^7 16S rRNA gene copies per reaction mixture. The techniques could detect up to 10^7 competing organisms. The limit of detection for the genus-specific probes ranged from 10^1 to 10^2 16S rRNA gene copies per reaction mixture. All probes performed well, with correlation (*R*) values of >0.99 for the standard curve and PCR efficiencies of 80%.

Contamination by human DNA. To assess the level of contamination of the skin swab material by human DNA, we performed qPCR for a human housekeeping gene (*GAPDH*) which is present in all nucleated cells of human origin (10). The *GAPDH* qPCR was performed in parallel with the bacterial qPCRs. In total, for each site, the log₁₀ mean *GAPDH* amplicons per μl of sample ranged from 0.07 ± 0.03 (forearm) to 2.14 ± 0.20 (behind ear) (Fig. 1 and Table 2). In general, the standard deviations were low across the 8 to 16 specimens sampled per site, indicating that the variation was site specific, rather than individual specific or technique related. The highest *GAPDH* values were obtained in specimens from sebaceous sites on the head (behind the ear and on the forehead) and were significantly greater than from the other dry sites (elbow, forearm, and foreleg) or moist sites (axilla) (*P* < 0.001).

Quantitative PCR detection of all bacteria and fungi in human skin samples. Real-time PCR analyses were performed to quantify all bacteria and fungi in the human skin samples obtained from 11 body sites (5 bilateral and 1 in the midline). All 82 samples studied from human skin showed 10^3 to 10^7 amplicons of bacterial 16S rRNA genes per extraction sample. The enumeration of the total bacteria, as well as four bacterial genera in the six cutaneous locales, is shown in Table 2 without
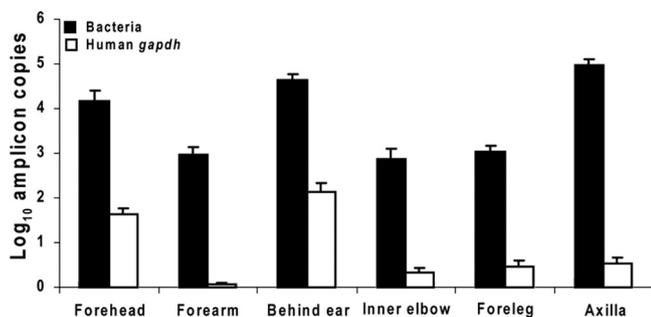
FIG. 1. Representation of bacterial and human DNA in skin swab specimens. For each of six assayed locales, skin swab specimens from 8 (forehead) to 16 (all other locales) were obtained, and qPCR was performed to detect human *GAPDH* or 16S rRNA using universal bacterial primers. From each site, bacterial amplicons outnumbered human amplicons by >2 $\log_{10}/\mu$l.

or with normalization to GAPDH levels. Before normalization, the highest bacterial $\log_{10}$ levels were found in the axilla (4.98 ± 0.13 $\log_{10}/\mu$l), which were substantially greater than the levels observed in the sebaceous sites (4.16 to 4.62 $\log_{10}/\mu$l) ($P = 0.03$). Levels at dry sites were even lower (~3 $\log_{10}/\mu$l) ($P < 0.001$). Using normalization, based on the number of copies of human *GAPDH*, the highest level of 16S bacterial rRNA again was found in the axilla (4.44 ± 0.18 $\log_{10}/\mu$l), and the level was >2 $\log_{10}$ higher than the levels for the other five body locations tested. The levels among the other five locales were similar to one another (range, 2.48 to 2.89 $\log_{10}/\mu$l). Before normalization, the range for fungal DNA was narrow (means, 1.48 to 2.79 $\log_{10}/\mu$l), with the highest levels found from the sebaceous sites on the head and with lower levels on the forelegs, forearms, and inner elbows; values for the forehead and behind the ears were virtually identical. After normalization to *GAPDH*, the highest level of fungi also was found in axillae (1.68 ± 0.32 $\log_{10}/\mu$l), with the lowest level found behind the ears (0.64 ± 0.20 $\log_{10}/\mu$l). In total, these results

indicate substantial quantitative differences in overall bacterial and fungal populations that are site specific.

Representation of major genera in human cutaneous samples. Results from our prior studies, based on analysis of ribosomal genes from clone libraries, provided evidence that four bacterial genera (*Corynebacterium*, *Streptococcus*, *Staphylococcus*, and *Propionibacterium*) and one fungal genus (*Malassezia*) were the most common genera on the human forearm (12, 25). Our present results show that the four bacterial genera represent 31.3 ± 9.3% (mean ± standard deviation [SD]) of the bacterial copies in the forearms (Fig. 2A and B), confirming their dominance among the hundreds of genera identified (5, 12, 15). *Corynebacterium* spp. were prominently found on the axilla and forehead (Fig. 2B), but there was substantial variation between individuals (range, ≪1.0 to 5.08 $\log_{10}/\mu$l in the axilla and ≪1.0 to 3.86 $\log_{10}/\mu$l on the forehead). More consistently low numbers were detected in other body locales. The specimens from the axilla and from the head (behind the ear and the forehead) harbored more *Streptococcus* spp. than did other areas. In total, *Streptococcus*, *Propionibacterium*, and *Staphylococcus* populations were variable in the different locations but to a lesser extent than was *Corynebacterium* (Fig. 2B). The proportions of the four genera in the axilla in relation to the total counts were significantly higher than those on the forearm, behind the ear, or the foreleg ($P < 0.01$). *Malassezia* spp. was calculated to account for 53% to 80% of all fungi in the different body locations; the highest numbers (2.74 ± 0.14 $\log_{10}/\mu$l) and highest proportions (80%) were found behind the ear (Table 2 and Fig. 3).

Comparison of lateral symmetries of the cutaneous human microbiota. We studied the symmetry of the left and right sides by comparing the patterns of five genera in the five body locales for which bilateral sampling was done. There was pronounced left/right symmetry at all locations ($r = 0.97$ and 0.94 for bacteria and fungi, respectively) (Fig. 4).

Comparison of intra- and interpersonal patterns of four major cutaneous bacterial genera. Figure 5 shows the propor-

TABLE 2. Composition of human skin microbiota from six cutaneous locations

Data treatment and body location	No. of samples	Mean $\log_{10}$ copies/μl ± SEM							
		All bacteria	*Corynebacterium*	*Streptococcus*	*Staphylococcus*	*Propionibacterium*	All fungi	*Malassezia*	Human *GAPDH*
Nonnormalized data									
Forehead	8	4.16 ± 0.25	0.77 ± 0.50	3.32 ± 0.22	2.62 ± 0.23	2.65 ± 0.19	2.79 ± 0.22	2.71 ± 0.31	1.62 ± 0.16
Forearm	16	2.97 ± 0.17	0.06 ± 0.06	2.00 ± 0.13	1.84 ± 0.26	1.85 ± 0.22	1.70 ± 0.14	1.32 ± 0.22	0.07 ± 0.03
Behind ear	16	4.62 ± 0.14	0.60 ± 0.32	3.76 ± 0.17	3.57 ± 0.14	3.56 ± 0.14	2.78 ± 0.14	2.74 ± 0.14	2.14 ± 0.20
Inner elbow	16	2.88 ± 0.22	0.08 ± 0.06	1.92 ± 0.20	1.87 ± 0.31	1.79 ± 0.29	1.59 ± 0.13	1.21 ± 0.22	0.33 ± 0.10
Foreleg	16	3.02 ± 0.14	0.35 ± 0.13	1.97 ± 0.11	2.08 ± 0.23	2.00 ± 0.21	1.48 ± 0.07	1.06 ± 0.14	0.46 ± 0.13
Axilla[a]	10	4.98 ± 0.13	2.98 ± 0.68	4.16 ± 0.17	4.13 ± 0.14	3.95 ± 0.14	2.22 ± 0.24	2.11 ± 0.31	0.55 ± 0.13
Normalized with respect to human *gapdh*									
Forehead	8	2.54 ± 0.21	−0.85 ± 0.53	1.70 ± 0.19	1.00 ± 0.20	1.02 ± 0.22	1.17 ± 0.20	1.09 ± 0.27	
Forearm	16	2.89 ± 0.17	−0.02 ± 0.05	1.92 ± 0.13	1.77 ± 0.26	1.77 ± 0.22	1.62 ± 0.14	1.25 ± 0.22	
Behind ear	16	2.48 ± 0.19	−1.54 ± 0.28	1.62 ± 0.19	1.43 ± 0.20	1.42 ± 0.18	0.64 ± 0.20	0.60 ± 0.22	
Inner elbow	16	2.56 ± 0.19	−0.25 ± 0.08	1.59 ± 0.18	1.54 ± 0.26	1.46 ± 0.23	1.26 ± 0.10	0.88 ± 0.19	
Foreleg	16	2.56 ± 0.21	−0.11 ± 0.20	1.51 ± 0.19	1.63 ± 0.31	1.54 ± 0.29	1.02 ± 0.13	0.60 ± 0.17	
Axilla[a]	10	4.44 ± 0.18	2.43 ± 0.75	3.62 ± 0.21	3.58 ± 0.20	3.40 ± 0.19	1.68 ± 0.32	1.57 ± 0.39	

[a] Data were not available from three subjects.
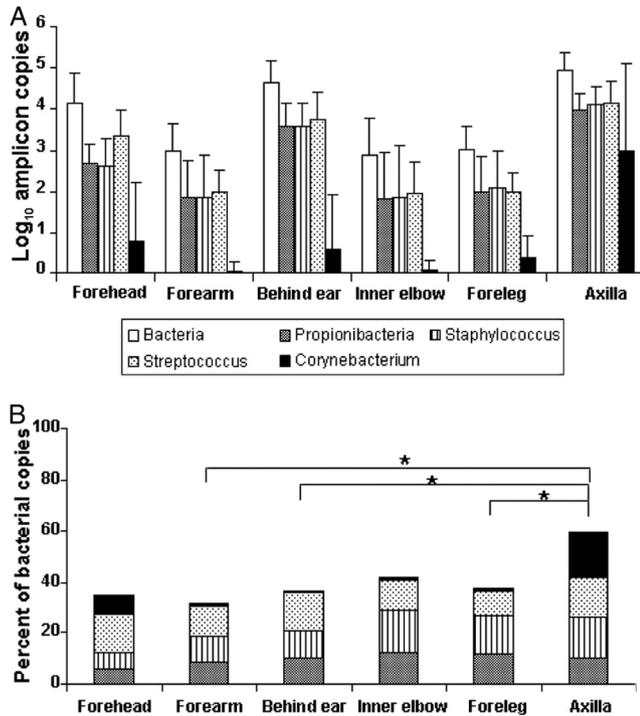[b] Data were normalized by dividing by the number of *GAPDH* amplicon copies.

FIG. 2. Enumeration of major bacterial genera in human cutaneous samples at six locales. (A) Log$_{10}$ amplification of total bacteria and four prominent cutaneous genera: *Propionibacterium*, *Staphylococcus*, *Streptococcus*, and *Corynebacterium*. The data were not normalized with respect to the human *GAPDH* gene. (B) Four bacterial genera as a proportion of total bacterial amplicons in six human cutaneous locales. An asterisk indicates that proportions are significantly ($P < 0.01$) different from other sites. For *Corynebacterium*, there was substantial interhost variation (from <1% to 45%).

tions of four major bacterial genera in eight different subjects. Overall, the patterns from the six locales showed extensive intrahost variation ($P < 0.001$, one-way ANOVA test). The patterns in the same locales but from different individuals also differed substantially. Significant interindividual differences were observed in the samples from forearms ($P = 0.004$), inner
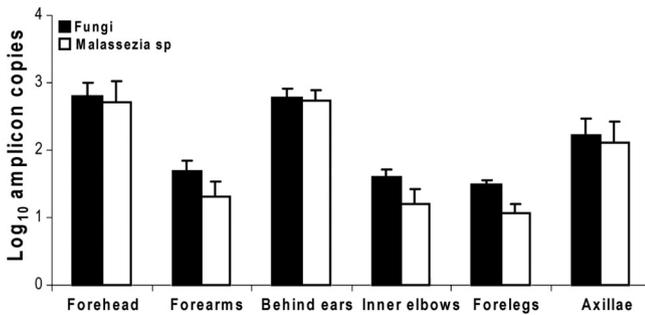

FIG. 3. Enumeration of fungal species in six cutaneous locales. From the eight tested subjects, a single specimen was obtained from the forehead and bilateral specimens were obtained from forearm, behind ear, inner elbow, and foreleg; samples from axillae were obtained for five subjects only. qPCR was performed using primers for universal fungal or *Malassezia* species. Bars indicate mean ± SD of the values across the subjects. The data were not normalized with respect to the human *GAPDH* gene.
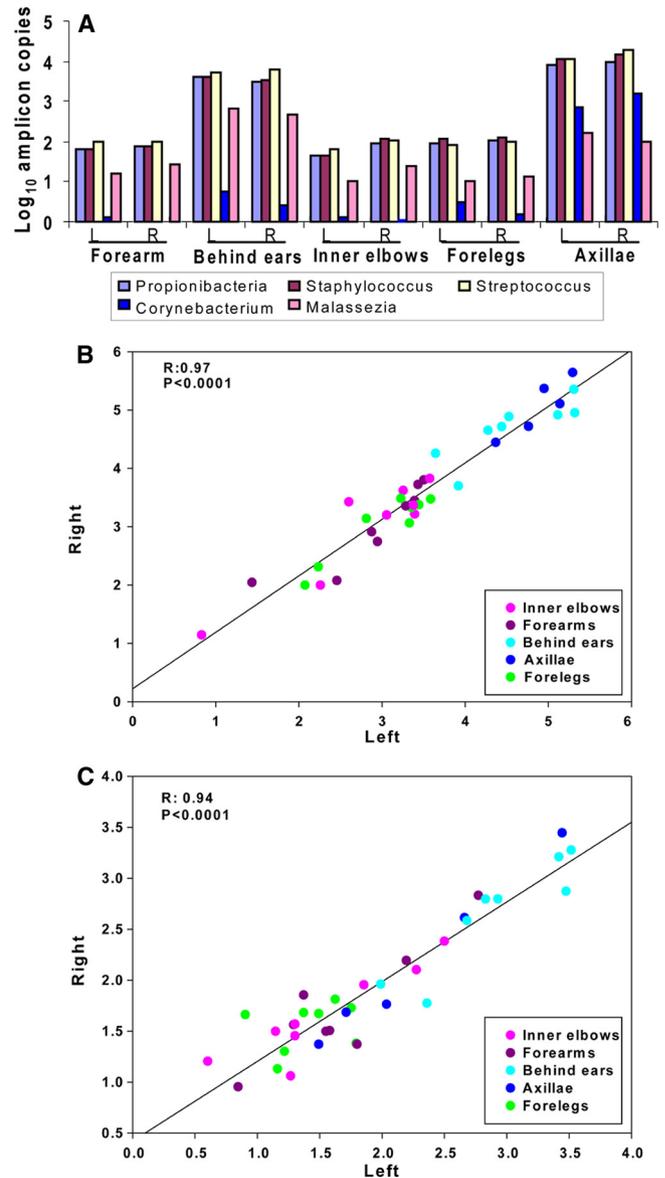
FIG. 4. Lateral symmetry of major microbial populations at five cutaneous locales in eight subjects, based on qPCR determination. (A) Genus-level qPCR results for five cutaneous locales. Values shown are mean log$_{10}$/µl for eight subjects (five subjects for axilla samples). The genera include *Propionibacterium*, *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Malassezia*. (B) Linear regression analysis of lateral symmetry of bacterial enumeration at five cutaneous locales, by individual study site. (C) An analysis similar to that in panel B, except universal fungal primers for qPCR were used.

elbows ($P < 0.001$), axilla ($P = 0.003$), and forelegs ($P = 0.002$). The composition patterns of the four bacterial genera were similar among different individuals ($P > 0.05$) in the samples from the head (behind the ear and the forehead). Analysis of variance also was performed across all of the specimens to estimate the effects of subject, site, and side on the overall variation (Table 3). For all four major bacterial genera, differences in site accounted for the greatest amount of variation, which was significant ($P < 0.01$) in each case. Although estimates were lower, the amount of variance attributable to
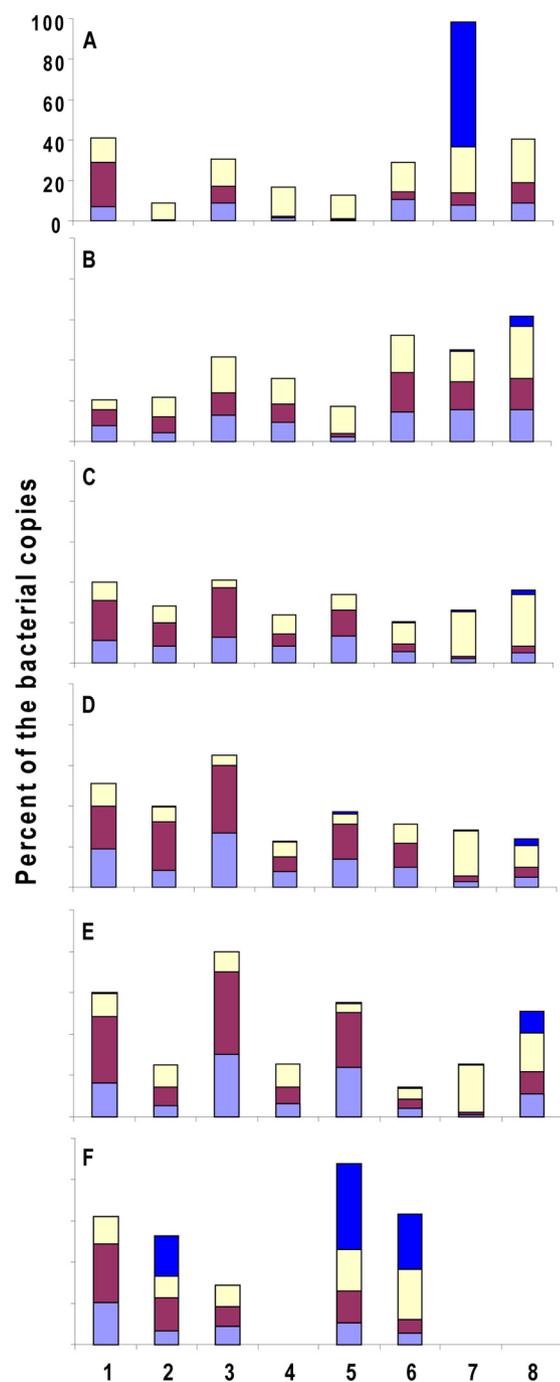
FIG. 5. Representation of four bacterial genera at six cutaneous sites in eight healthy subjects. Numbers 1 through 8 designate the enrolled subjects. (A) Forehead; (B) behind left and right ears; (C) left and right forearms; (D) left and right forelegs; (E) left and right inner elbows; (F) left and right axillae. For three subjects, no axillary specimens were included. The bacterial genera are color coded as for Fig. 4A.

between-subject differences also was significant for all genera except for *Corynebacterium*. Exploratory analyses also were conducted to assess pair-wise interactions between subjects, site, and time. The data indicated significant interactions between subjects and site for all four major bacterial populations

($P < 0.01$). This finding provides evidence that the relationship between subjects and the level of major bacterial populations varies by site.

Fluctuation of the four bacterial genera in two subjects. Skin samples were collected 1 month apart from two healthy subjects to examine variations in the four major bacterial genera (Fig. 6 and 7). The results of cluster analysis showed that samples from the same subject clustered closely, whereas the two time points were not clustered. Numbers of *Streptococcus* and *Staphylococcus* were significantly different for samples obtained 1 month apart, with fluctuating proportions of the two genera ($P < 0.001$ and $P = 0.001$, respectively). For *Corynebacterium* and *Propionibacterium*, time of sampling had little effect on the overall variation, whereas it was the dominant source of variation for *Staphylococcus* and *Streptococcus*. The amount of variation in the level of staphylococci and streptococci that is attributable to time (33.2% and 76.6% for staphylococci and streptococci, respectively) is greater than the variation due to subject, side, or site (all <25% and <1% for staphylococci and streptococci, respectively) (Table 3).

DISCUSSION

To investigate the population structure of the human cutaneous microbiota, a set of primers and TaqMan MGB probes was adapted or designed, validated, and deployed to enumerate the predominant bacterial and fungal genera in several cutaneous locales from healthy human subjects. The results show that the qPCR assays are sensitive, rapid, and reproducible methods for the quantitation of the major microbial genera that populate human skin.

For accurate comparison of bacteria and fungi in different samples, the amount of total host DNA can provide a measure to facilitate standardization. Since the *GAPDH* gene is a conserved human housekeeping gene used to normalize gene expression data (2), a *GAPDH* qPCR was used to standardize the process of sampling. The relatively high numbers of 16S rRNA bacterial copies in relation to the copies of human *GAPDH* that we observed demonstrate that skin swabbing captures a population of DNA highly enriched for microbiota. The amount of interindividual variation at each specific locale was low, indicating the relative uniformity of the swabbing procedure. Our data suggest that standardization is site specific and differs for sebaceous locales in comparison to dry sites, which have smaller amounts of human DNA. Although further study is needed, site specificity may be presumed for now.

The results from prior studies (5, 9, 12, 13, 15, 25, 26), using analysis of ribosomal genes from clone libraries or high-throughput sequencing, provided evidence that four bacterial genera, *Corynebacterium*, *Streptococcus*, *Staphylococcus*, and *Propionibacterium* and one fungal genus, *Malassezia*, were the most common in the human forearm. Our observation that the total for the four bacterial genera was calculated to represent 31% to 59% of all the bacterial 16S rRNA copies in each of the different locales provides further confirmation of these four conserved major populations. That *Malassezia* spp. represented 53% to 80% of all the fungal RNA amplicons is consistent with our prior studies of the forearm using the clone library method (25) and provides further evidence that these

TABLE 3. Analysis of variance for the four major bacterial populations in the study subjects

Source of variance	*Corynebacterium*		*Propionibacterium*		*Staphylococcus*		*Streptococcus*	
	Estimate (95% CI[c])	P value	Estimate (95% CI[c])	P value	Estimate (95% CI)	P value	Estimate (95% CI)	P value
Total variance explained[a]	45.0 (27.0–59.0)		69.0 (57.0–77.0)		68.0 (56.0–77.0)		76.0 (0.7–0.8)	
Variance explained by:								
Subjects	5.5 (0.0–16.7)	0.05	18.8 (1.6–30.6)	<0.01	21.6 (3.5–33.7)	<0.01	7.5 (0.0–15.6)	<0.01
Site	41.4 (25.2–54.8)	<0.01	41.4 (23.8–53.7)	<0.01	36.6 (18.9–49.5)	<0.01	58.2 (42.9–67.6)	<0.01
Side	0.5 (0.0–6.2)	0.57	0.3 (0.0–5.3)	0.59	0.2 (0.0–6.0)	0.48	0.0 (0.0–6.3)	0.37
Subsample with repeated measurements[b]								
Total variance explained	39.0 (6.0–63.0)		78.0 (59.0–87.0)		75.0 (52.0–85.0)		74.0 (51.0–85.0)	
Variance explained by:								
Subjects	11.8 (0.0–37.5)	0.02	34.4 (7.8–55.9)	<0.01	14.9 (0.0–39.3)	<0.01	0.9 (0.0–0.13)	0.96
Site	29.6 (4.5–53.9)	<0.01	25.7 (7.6–47.8)	<0.01	24.8 (0.5–47.3)	<0.01	0.0 (0.0–14.7)	0.38
Side	2.1 (0.0–11.0)	0.82	1.4 (0.0–20.9)	0.11	0.6 (0.0–13.8)	0.56	0.8 (0.0–10.0)	0.76
Time	0.9 (0.0–18.6)	0.45	6.0 (0.0–28.8)	0.01	33.2 (7.1–55.1)	<0.01	76.6 (59.2–85.6)	<0.01

[a] Absolute values ($\log_{10}$ copies/μl) for bacterial density used for this analysis.
[b] Ratio of specific bacteria to total bacteria used for this analysis.
[c] CI, confidence interval.

organisms represent the most frequent fungal genus at the tested locales.

Many culture-based studies have reported that the composition of the normal bacterial biota varies according to body location (11, 21, 22, 29). Our results demonstrated that the
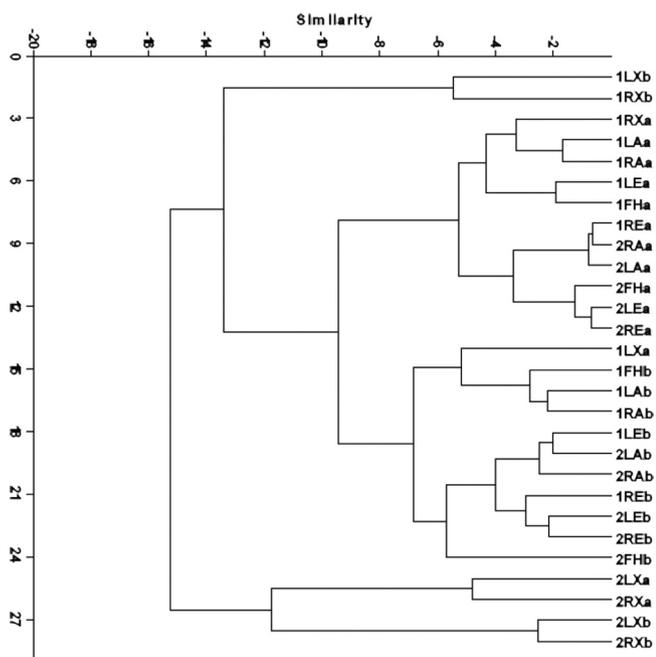

FIG. 6. Clustered display of data from repeat sampling of four bacterial genera in two subjects. The distribution of four bacterial genera from two healthy subjects (subjects 1 and 2) at two time points is represented by a dendrogram based on UPGMA cluster analysis. The samples are coded as follows: 1, subject 1; 2, subject 2; a, first sampling; b, second sampling; FH, Forehead; LX, left axilla; RX, right axilla; LA, left forearm; RA, right forearm; LE, behind left ear; RE, behind right ear.

axilla harbored the highest density of bacterial copies and the highest percentage of the four most common bacterial genera. Although about 1% of the total bacterial population present in all sites of all subjects was *Corynebacterium* spp., this genus was most highly represented in the axilla, whereas *Streptococci* spp. were present at higher concentrations than the other three bacterial genera on the forehead and behind the ears. Such data extend our knowledge of the site specificity of the distribution of bacterial species in human skin and point to a means for further genus-specific explorations.

To examine changes in the numbers of the four bacterial genera in the skin samples over time, we collected samples from two of the subjects at intervals 1 month apart as a pilot study. The results showed that the total levels of the four common major genera of the normal human skin biota (3, 11) did not change greatly, but that their proportions did (Fig. 6 and 7). The substantial changes in the proportions of the genera *Streptococcus* and *Staphylococcus* over time suggest that these might be highly dynamic (Fig. 6 and 7) and should be followed in larger studies. The high correlations observed across sides for each site show that the human skin microbiota has a high level of left/right symmetry, as expected (9, 12, 25); the high correlations also help validate our qPCR findings. Over time, as indicated by the resampling, the host-specific conservation persists, but without a strong relationship to the prior sample from the same site. These observations indicate that the major bacterial biota is dynamic (primarily reflecting the *Streptococcus* and *Staphylococcus* populations) over the 1-month period sampled and that the changes influenced both sides similarly.

In conclusion, we developed quantitative PCR methods to investigate the distributions of the four most common bacterial genera and one fungal genus in different human cutaneous sites. The data reported here extend previous observations (5, 9, 12, 13, 15, 25) and provide further evidence that the distributions of the major bacterial and fungal genera are both site and host specific. As the Human Microbiome Project (16, 27)
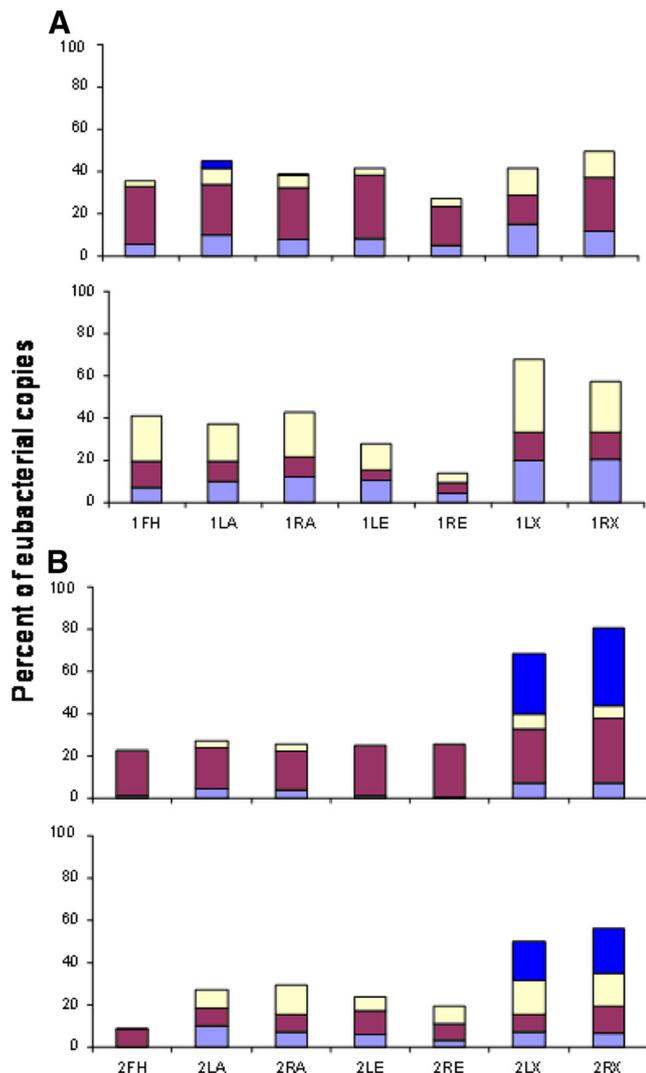
FIG. 7. Distribution of four bacterial genera from two subjects at two time points. (A) Proportion of the total bacterial amplicons represented by the four bacterial genera from subject 1 at two time points. The bacterial genera are color coded as for Fig. 4A. The bars are coded as follows: FH, forehead; LA, left forearm; RA, right forearm; LE, left behind ear; RE, right behind ear; LX, left axilla; RX, right axilla. The data were not normalized with respect to the human *GAPDH* gene. (B) Results for subject 2, with color coding and abbreviations as for panel A.

progresses, quantitative PCR will enable more-detailed analyses of target taxons of the human cutaneous microbiota, complementing high-throughput sequencing.

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