Quantification of the Human Skin Microbiota

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Abstract

- Understanding how quantities of skin-associated bacteria vary across different body habitats is vital to understanding their ecology and role in affecting human health
- How does the total number of bacteria counted vary between dry, moist, and sebaceous skin types?
- How does the proportion of live and dead bacteria vary between dry, moist, and sebaceous skin types?

Summary of Results			
	Dry	Moist	Sebaceous
Colony counts (CFU/cm^2)	24	171	626
qPCR (# of amplicons per well)	2.65 x 10^4	2.82 x 10^4	2.90 x 10^4
Fluorescence microscopy (cells/cm^2; % viability)	1.12 x 10^7; 14.0	1.31 x 10^7; 15.0	3.29 x 10^7; 6.78

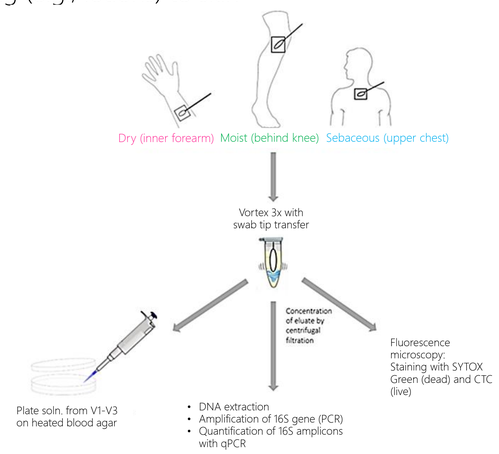
Background

- Skin: primary interface for human-microbe interaction
- Lack of research on absolute bacterial abundance on human skin
- No published data on viability of skin-associated bacteria
- Distinct microenvironments at dry, moist, and sebaceous skin sites likely affect numerical patterns of diversity[3]
- Method of quantification likely to affect # of bacteria counted:

	Method of Quantification		
	Colony counts	qPCR of 16S gene	Fluorescence microscopy
Benefits	Easy, fast, inexpensive	Facilitates amplification of most bacterial DNA in sample	Cells can be directly counted and stained for certain properties
Limitations	Colonies counted represent only viable, culturable cells Only ~1% of all bacterial species are culturable in lab	Indirect method of quantification Cannot distinguish between DNA from live and dead cells	Visibility of cells may be reduced due to limits of dyes or properties of cells Time consuming

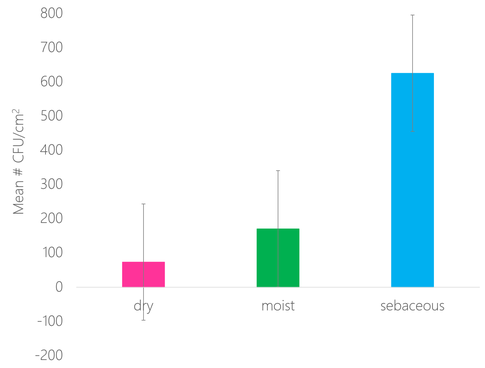
Methods

- Participants: 8 female, 6 male adults
- 12 hours before sampling: no bathing/swimming or applying anything (e.g., lotions) to skin



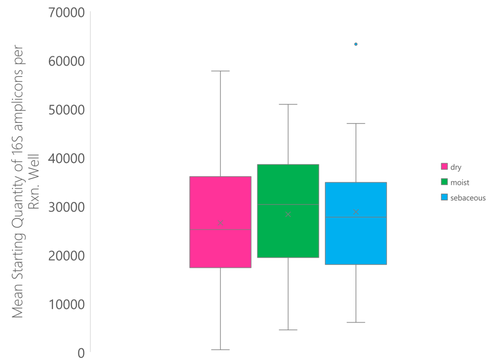
IRB-approved protocol #06082013.012

Results



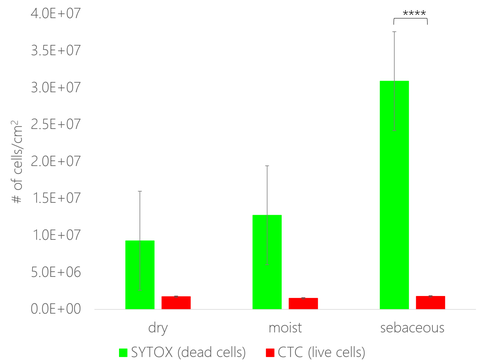
No significant differences in cellular abundance between dry, moist, and sebaceous sites, estimated from colony counts

Figure 1. Estimates for the mean # CFU/cm^2 skin sampled determined from colony counts. Error bars: standard error. No statistical significance determined from one-way ANOVA: $F_{2,39}$=1.68, p=0.20.



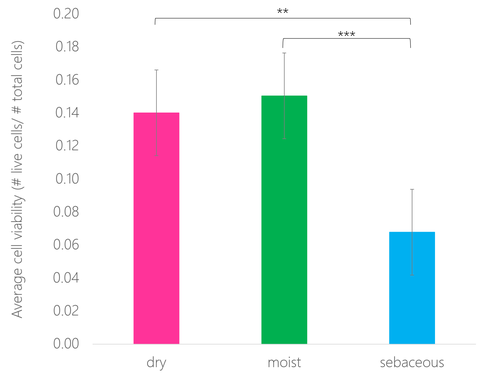
No significant differences in 16S gene copy number between dry, moist, and sebaceous sites, determined from qPCR

Figure 2. Mean starting quantity of 16S amplicons per qPCR reaction well. Due to multiple amplification steps, we could not confidently use qPCR data to extrapolate the number of cells/cm^2 on the skin. Error bars: standard error. No statistical significance determine from one-way ANOVA: $F_{2,38}$=0.081, p=0.92.



The quantities of dead cells and live cells found at sebaceous skin sites are significantly different

Figure 3. Estimates for mean # of cells/cm^2 skin sampled determined from fluorescence microscopy. Error bars: standard error. Significant difference was found between estimates of live and dead cells for sebaceous sites from multiple comparisons ANOVA, $p << 0.05$.



Significant differences in cell viability were determined between dry and sebaceous and moist and sebaceous sites

Figure 4. Mean cell viability. Cell viability was determined by dividing the # of live cells counted by the total number of cells counted (live + dead). Error bars: standard error. Statistical significance between dry and sebaceous (p=0.0046) and moist and sebaceous sites (p=0.00075) was determined from two-tailed t-test.

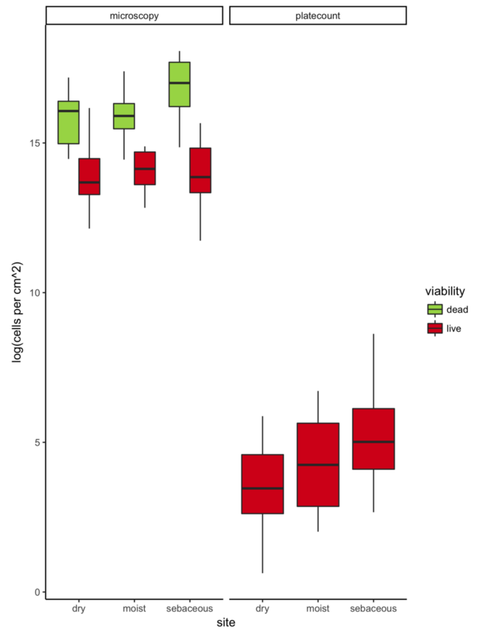


Figure 5. Comparison of cell density estimates made from colony counts and fluorescence microscopy. Estimates have been log transformed to be able to represent them on the same axis. Statistical significance between estimates made from colony counts and microscopy was determined from multiple comparisons ANOVA, $p << 0.05$. Combined data from these methods showed that abundance is greater at sebaceous sites than moist (p=0.012) and dry (p=0.0061) sites. Error bars: 95% confidence intervals.

Discussion

- Colony counts: lower estimates of cell density than what has already been determined from other culture-based studies[4]
  - Why? Colonies only represent the cells that are live and culturable, more colonies could have developed with use of other media and culture conditions.
- qPCR: useful for relative comparisons between sample types but multiple amplification steps prevented us from making confident estimates for cells/cm^2
  - Next time: do qPCR of genomic DNA, rather than of 16S amplicons, to be able to make more confident estimates of cell density
- Fluorescence microscopy: provided estimates of abundance for skin-associated bacteria that have been corroborated by both culture-dependent and culture-independent studies[1,4]
  - Fluorescent dyes CTC and SYTOX Green allowed us to determine that ~5-15% of the skin microbiota is actively respiring
  - Next time: use DAPI stain to determine which cells are alive but not actively respiring (dormant), which may be important for maintaining genetic diversity and nutrient cycling[2]
- Shown here is the first study comparing multiple methods for quantifying the skin microbiota, including an assay for viability

References & Acknowledgements

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2. Lennon, Jay T., and Stuart E. Jones. "Microbial seed banks: the ecological and evolutionary implications of dormancy." Nature Reviews Microbiology 9.2 (2011): 119-130.
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