Next-Generation Sequencing and Culture-Based Techniques Offer Complementary Insights into Fungi and Prokaryotes in Beach Sands

Christopher Staley[a], Daniela Romão[b], Filipa Ferreira[b], Raquel Rodrigues[b], Raquel Sabino[c], Cristina Veríssimo[c], Ping Wang[a], João Brandão[b], and Michael J. Sadowsky[a,d]

•[a]BioTechnology Institute, University of Minnesota; [b]Water and Soils Unit – Department of Environmental Health, National Institute of Health Dr Ricardo Jorge;

•[c]Reference Unit for Parasitic and Fungal Infections – Department of Infectious Diseases, National Institute of Health Dr Ricardo Jorge; [d]Department of Soil, Water, and Climate, University of Minnesota

Contact:
1479 Gortner Ave, Suite 140
St. Paul, MN 55108
612-625-1722
cmstaley@umn.edu

Introduction

- Beach sands represent reservoirs for a variety of bacterial, fungal, and protozoan pathogens.[1]
- Epidemiological studies and monitoring efforts have only recently been explored to protect public health.[1]
- Current monitoring efforts are based on culture-based enumeration of fecal indicator bacteria and selected fungal pathogens.[2]
- Next-generation sequencing (NGS) methods are increasingly being used to more completely characterize microbial communities in sands.[3]
- Taken together, NGS and conventional methods can be used in a tool-box approach to better assess health risks.[4]

We hypothesized that NGS and culture-based methods would identify the same, predominant taxa in beach sands:

- *Culture-based methods will show greater sensitivity to low-abundance potential pathogens*
- *NGS will offer more extensive characterization of the community and identify novel targets for monitoring efforts*

Methods

- Composite backshore sands collected from 4 regions in Portugal, 6 beaches from Apr – Nov 2013
- Yeasts, molds, and dermatophytes enumerated by culture followed by classic identification methods
- Fecal indicator bacteria (FIB) enumerated by IDEXX MPN
- Illumina NGS targeting V4 (prokaryotes) and ITS1 (fungi)

References

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3. Staley C and Sadowsky MJ. 2016. Appl Environ Microbiol 82: 2751-62
4. Ahmed W, Staley C, Sadowsky MJ, *et al.* 2015 Appl Environ Microbiol 81: 7067-77
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Results

Fungal Characterization

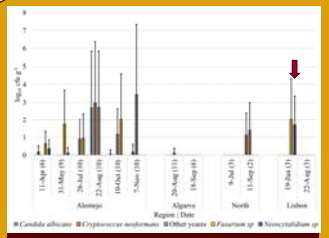

Fig. 1 - Average concentrations of fungi enumerated by cultured-based methods. *n* shown in parentheses.

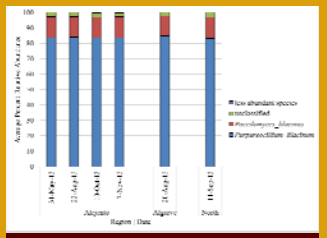

Fig. 2 - Distribution of species characterized by NGS.

- Low culturable fungal densities
  - No differences by region, date
  - Except *Neoscytalidium* ($P \leq 0.003$)
- *Epidermophyton* cultured in Northern region (0.22 ± 0.33 $\log_{10}$ cfu g^{-1})
- About 960 – 1200 OTUs sequenced/sample
- Shannon indices ≤ 2.00
- Communities primarily comprised of *Purpureocillium lilacinum* and *Paecilomyces lilacinus* by NGS
  - Now considered same sp.
- Other spp. < 0.06% of reads
- No NGS differences by region, date (ANOSIM $P = 1.00$)

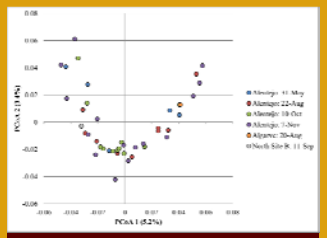

Fig. 3 - Principal coordinate analysis of Bray-Curtis dissimilarities ($r^2 = 0.032$).

Table 1 – Correlations between culture-based and NGS fungi

Culture	NGS	Spearman's ρ (*P*)
Total filamentous fungi		0.382 (0.013)
Mircosporum sp.	*Sordariomycetes_spp*	-0.308 (0.047)
Total dermatophytes		-0.388 (0.012)
Candida albicans		0.303 (0.033)
Other yeasts	Unclassified fungi	0.363 (0.019)
Total yeasts		0.341 (0.028)
Candida albicans	*Cryptococcus*	0.543 (< 0.001)
Fusarium spp.	*Rhodotorula, Chaetomium, Myceliophthora*	0.548 - 0.592 (< 0.001)
Microsporum	*Phaeoacremonium*, Unclassified *Mycosphaerellaceae*	0.494 (0.001)

Bacterial Characterization

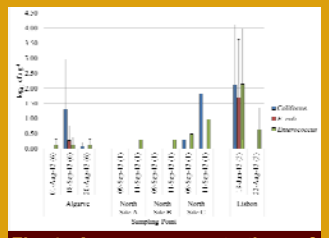

Fig. 4 - Average concentrations of indicator bacteria in samples characterized by culture-based methods. *n* shown in parentheses.

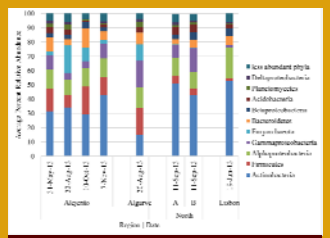

Fig. 5 - Distributions of abundant phyla in samples characterized by NGS.

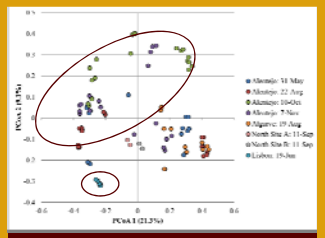

Fig. 6 – Principal coordinate analysis of Bray-Curtis dissimilarities ($r^2 = 0.56$).

- Concentrations of FIB low or not detected
  - All FIB were correlated with each other (r = 0.51-0.63)
- No differences by region, date
- About 260 – 2600 OTUs sequenced/sample
- Shannon indices ~5.00
- Up to 19% of communities were Euryarchaeota
- Significant differences in composition by region and date (ANOSIM $P \leq 0.037$)

Conclusions

A toolbox monitoring approach incorporating culture-based and NGS methods improves accuracy of microbial community characterization

- NGS characterization reveals pathogens are a minority in the fungal community.
  - *Purpureocillium lilacinum* is an infrequent human pathogen.[5]
- Current NGS fungal databases must keep pace with changing taxonomic designations.
  - Many fungi characterized by NGS remain unclassified in taxonomic databases.
- Archaeal abundances in sands have largely been ignored and represent an unknown health risk.