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Letters

Modern Methods for Delineating Metagenomic Complexity

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We appreciate the comments of Ackelsberg et al. (Ackelsberg et al., 2015) and have decided to revise the paper (Afshinnekoo et al., 2015) as follows:

> Figure 3B has been corrected to show the general coverage of the *Yersinia pestis* pMT1 plasmid, but not the murine toxin gene (yMT). The initial claim of "…consistent 20× coverage across the murine toxin gene…" was erroneously based on looking at annotations from related plasmids and comparing different reference sequences. In actuality no reads mapped to the yMT gene.
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> The result of low coverage to the *Bacillus anthracis* plasmids (pXO1 and pXO2) and no evidence of plcR SNP—an often defining feature of anthrax—is now reported in the Results section.
>
> The language in the Summary, Results, and Discussion has been revised, and speculative text about pathogenic organisms has been deleted. We now state that although all our metagenomic analysis tools identified reads with similarity to *B. anthracis* and *Y. pestis* sequences, there is minimal coverage to the backbone genome of these organisms, and there is no strong evidence to suggest these organisms are in fact present and no evidence of pathogenicity.

Furthermore, in regards to the concerns of the culture methods we have posted subsequent details on the study website (http://www.pathomap.org/2015/04/13/culture-methods/) and below.

A second culture experiment was performed to address the question of antibiotic resistance (Afshinnekoo et al., 2015, Figure 4A). Bacteria were cultured in LB agar and then spread onto LB plates, after lawn growth, single colonies were picked and then plated onto antibiotic plates (kanamycin – 50 ug/ml, chloramphenicol – 35 ug/ml, and ampicillin – 100 ug/ml) and growth was assessed. Plates were incubated at 37°C. As a control, air samples were taken and cultured at every location. In all cases, these did not yield growth. The non-selective plate done last when replica plating also serves as a control. There was no quantitative confirmation of bacterial versus non-bacterial organisms, although there was no observable fungal growth in the samples. Further experiments are being done to dive deeper into the question of viability of microorganisms on the subway system as well as the presence of antibiotic-resistant bacteria.

The field of metagenomics is relatively new but has great potential to serve an incredibly important role both in our understanding of the world around us—with key applications in the built environment—as well as the clinical realm. Nevertheless, there are still major hurdles and challenges that the field faces in order to realize this potential. We welcome and appreciate the discussion (http://microbe.net/2015/02/17/the-long-road-from-data-to-wisdom-and-from-dna-to-pathogen/) prompted by our study, and we anticipate that this large dataset will enable further experimentation, additional testing of taxonomic tools, and hopefully help in developing methodologies for metagenomic analysis.

REFERENCES

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