Use of propidium monoazide (PMA) followed by 16S rDNA sequencing in order to discriminate live and dead cells in surface samples from collective kitchens

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Introduction:

As humans in the industrial world are spending most of their lifetime indoors, the **"built environment" microbiota** has been defined as all microorganisms found inside buildings. Kitchens importantly contribute to this microbiota because of dynamic bacterial transfers between **food, utensils and food handlers**. **Collective kitchens** present a higher risk of microbial transmission, as they produce meals for greater populations with lower immune defences **(children, patients, elderly)**. These transmissions are usually tracked by culture-dependent microbiology. The recent advances in molecular biology permit to overcome the inherent limitation of sequencing techniques consisting in analysing all DNA present in a sample. Hence, sample treatment with **propidium monoazide (PMA)** prior to DNA extraction allows the binding of the substance to free DNA and also enters cells with compromised membrane and thus **PCR amplification of only viable cells.**

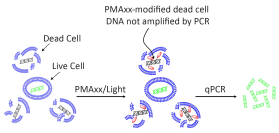

Figure 1. Mechanism of action of propidium monoazide (PMAxx).
It links covalently to free DNA or DNA from cells with compromised membranes.

⇒ Find viable bacterial populations in surface samples from collective kitchens

Material & Methods:

- 154 surface samples from 5 hospital and 4 school kitchens in Belgium
- Two DNA extractions of 1 sample: **One with & one without PMA treatment prior to extraction** (QIAGEN DNeasy)
- Sequencing of the region V1-V3 of the 16S rRNA gene (Illumina MiSeq)
- **58 pairs of samples** were curated by mothur v1.39.5 and aligned to the SILVA database v128
- The Good's coverage, Simpson's inverse diversity index and LEfSe (linear discriminant analysis (LDA) effect size) were performed on mothur v1.39.5
- Graphical representations were implemented on Prism 7

Results:

The **Good's non-parametric coverage** estimator: 98.4-99.9% => sufficient extent of sampling of the communities.
The **Simpson's inverse diversity index** => no significant difference in diversity between the two groups.

1) Viability index by $\log_{10}$(PMA/noPMA)

The genera ***Elizabethkingia* spp.**, ***Propionibacterium* spp.** and ***Escherichia-Shigella* spp.** were present in higher relative abundances in most PMA counterparts, indicating either their higher viability or contaminant nature (Figure 2).

Two-way ANOVA between the two groups showed that the variations in the relative abundance of *Escherichia* were due to the PMA treatment, whereas variations of *Elizabethkingia* and *Propionibacterium* were due not only to PMA treatment, but also depending on the nature of the sample ($p<0.05$).

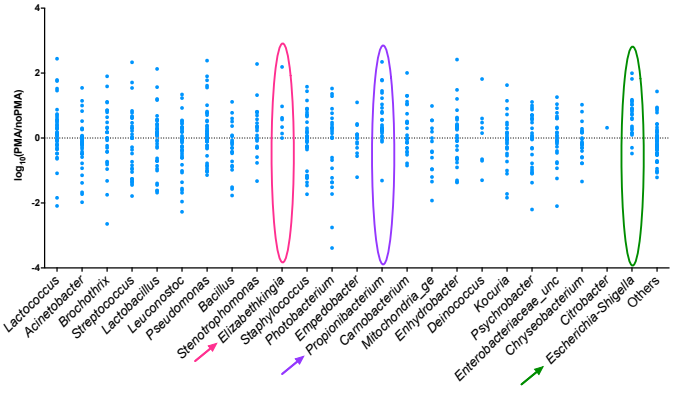

Figure 2. The $\log_{10}$(ratio) between relative population abundance of PMA-treated and untreated pairs of samples of the 24 most abundant genera. The remaining genera were classed in the 'Others' group. Larger numeric values indicate higher viability.

2) Discovery of metagenomic biomarkers

The LEfSe indicated that the genus ***Escherichia-Shigella*** could be associated with the **PMA** group and the genera *Acidovorax, Comamonas* and *Acidovorax* with the untreated groups. The LDA scores of these genera above 2 and were statistically significant ($p<0.05$, Figure 3 A). The average relative abundance of the *Escherichia-Shigella* genus was 1.02% in PMA-treated compared to 0.083% in untreated groups, whereas for the other genera the percentages were lower than 0.5% (Figure 3 B).

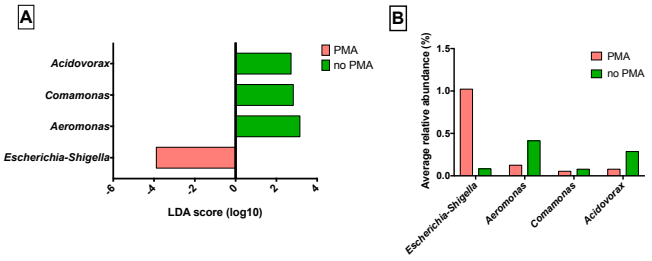

Figure 3. Discovery of metagenomic biomarkers by LEfSe.
A) These genera were significantly differentially abundant between the untreated and PMA-treated groups ($p<0.05$).
B) The average relative abundance of the genera in both groups

Conclusion & Perspectives:

The **bacterial concentration of surface samples** was a first limiting factor for this study (mean concentration 3.44 log(CFU/mL). Lower bacterial concentrations involve a risk of PCR amplification of contaminant DNA. Previous results from the laboratory showed higher relative abundances of *Elizabethkingia* in pure cultures lower than 2-3 log(CFU/mL), indicating its contaminant nature. *Escherichia* genus could be associated with PMA-treated groups, which can be either explained by a higher viability which was not detected by classical microbiology (only 2 + samples) or a contaminant source. *Propionibacterium* is mostly associated with human skin and could be viable indeed.
In the future, analyses with a higher number of pairs, separations on their nature and estimations of the DNA concentrations of the genera will be performed in order to confirm these trends.

References:

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Höppe, P., Martinac, I., 1998. Indoor climate and air quality. International Journal of Biometeorology 42, 1–7.
Figure 1: From: https://biotium.com/wp-content/uploads/2015/05/PMAxx-diagram.jpg (Accessed on 13th June, 2018).