Automatic metabolome profiling of bacterial colony heterogeneity by multimodal imaging with mass spectrometry and microscopy

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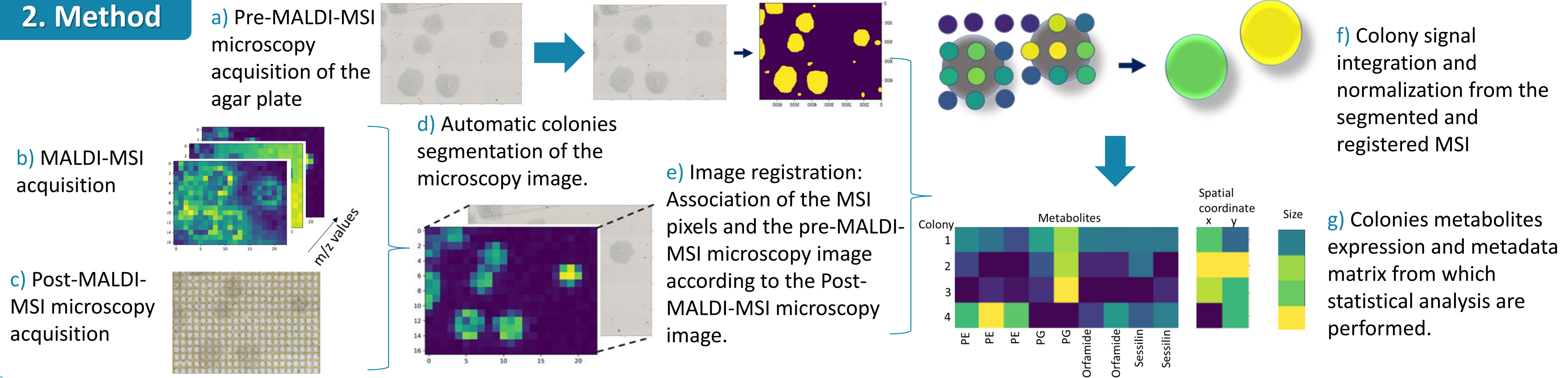
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1. Introduction

- Mass spectrometry imaging (MSI), used in the study of metabolites production in bacteria, is often limited to very few colonies.
- Taking inspiration from the work of Rappez et al [1], we developed a new technique to study the metabolite production of multiple bacterial colonies, from a combination of optical microscopy and MSI.
- We show the molecular diversity of *Pseudomonas sp. CMR12a* colonies on agar plates alone and when interacting with *Bacillus velezensis GA1*.

2. Method

a) Pre-MALDI-MSI microscopy acquisition of the agar plate

b) MALDI-MSI acquisition

c) Post-MALDI-MSI microscopy acquisition

d) Automatic colonies segmentation of the microscopy image.

e) Image registration: Association of the MSI pixels and the pre-MALDI-MSI microscopy image according to the Post-MALDI-MSI microscopy image.

f) Colony signal integration and normalization from the segmented and registered MSI

g) Colonies metabolites expression and metadata matrix from which statistical analysis are performed.

3. Results

- The method is performed on 2 conditions of plates: **control** where *CMR12a* is alone and **interaction** where *CMR12a* is in contact with *GA1*.

- The method has detected **962** *CMR12a* colonies from the different slides.

- Different signals were detected and annotated mainly as **lipids** and **lipopeptides**.
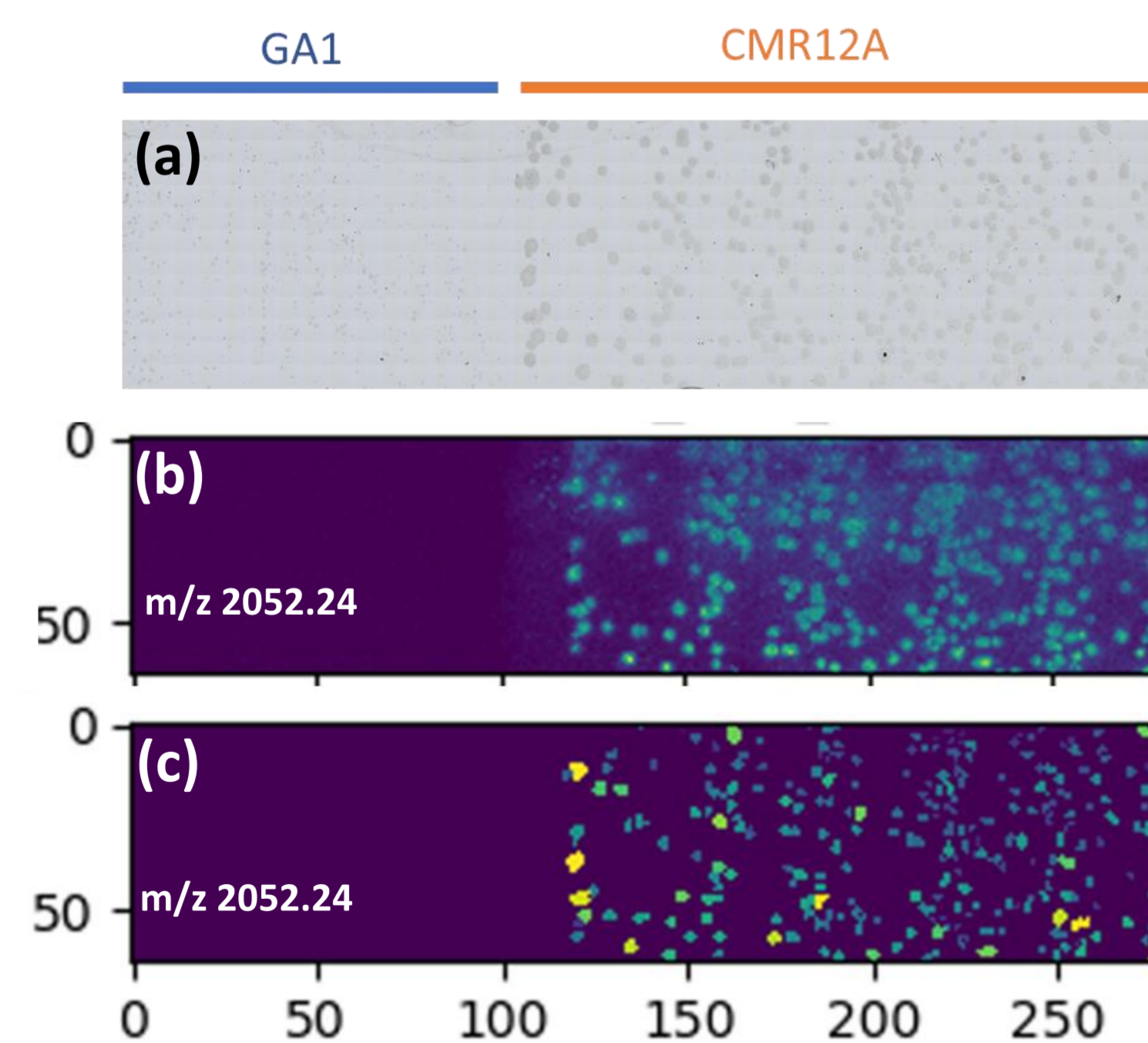
- Example of an **interaction** plate: (a) microscopy, (b) MS intensity of that single ion. (c) The estimated signal of an ion for each detected colony according to our method.
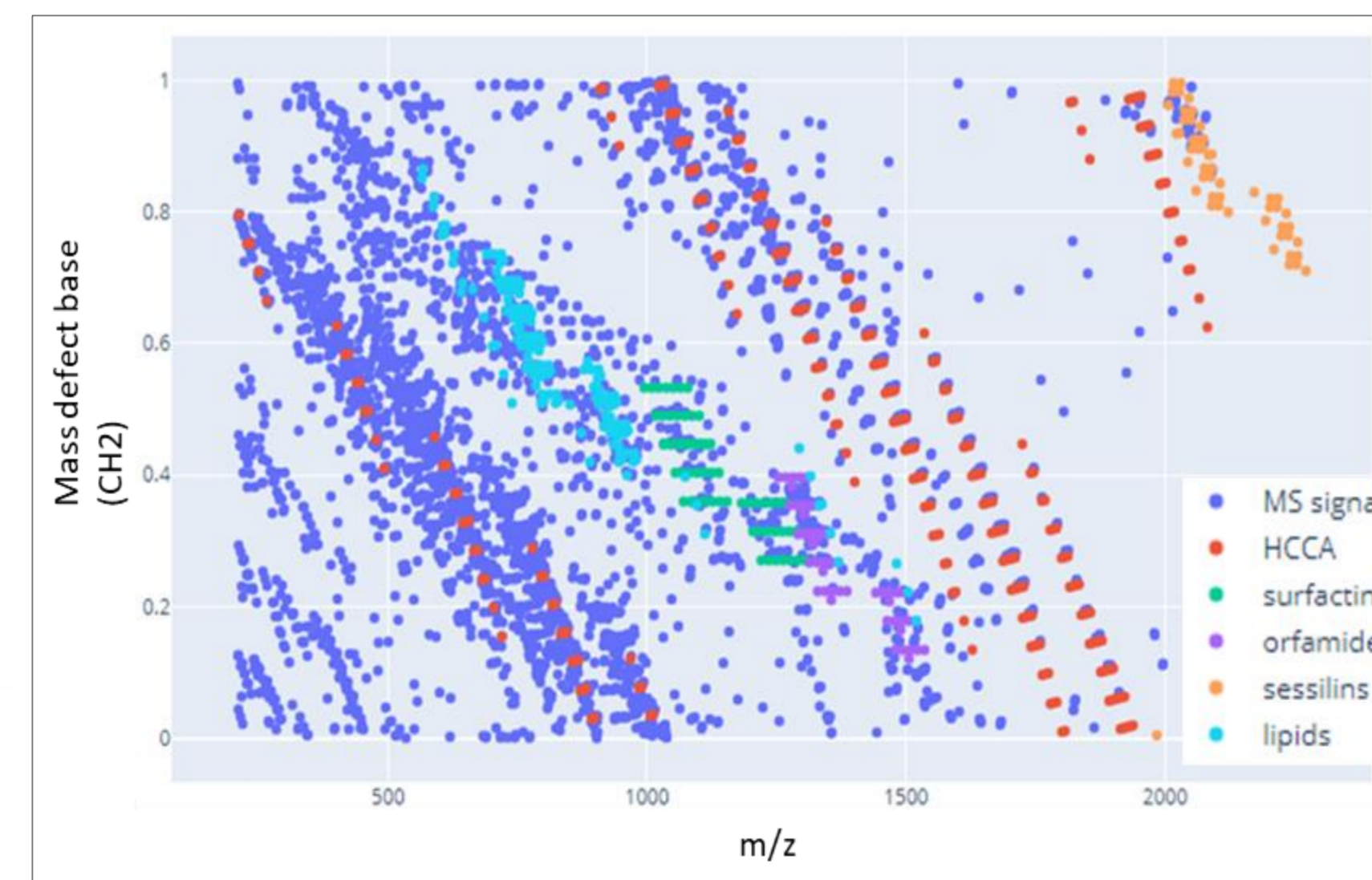
- Global Kendrick plot of the mean spectrum for the **interaction** plates.
- The detected lipopeptides (surfactins, sessilins and orfamides) and the lipids (mainly PE and PG) are highlighted in various colors.
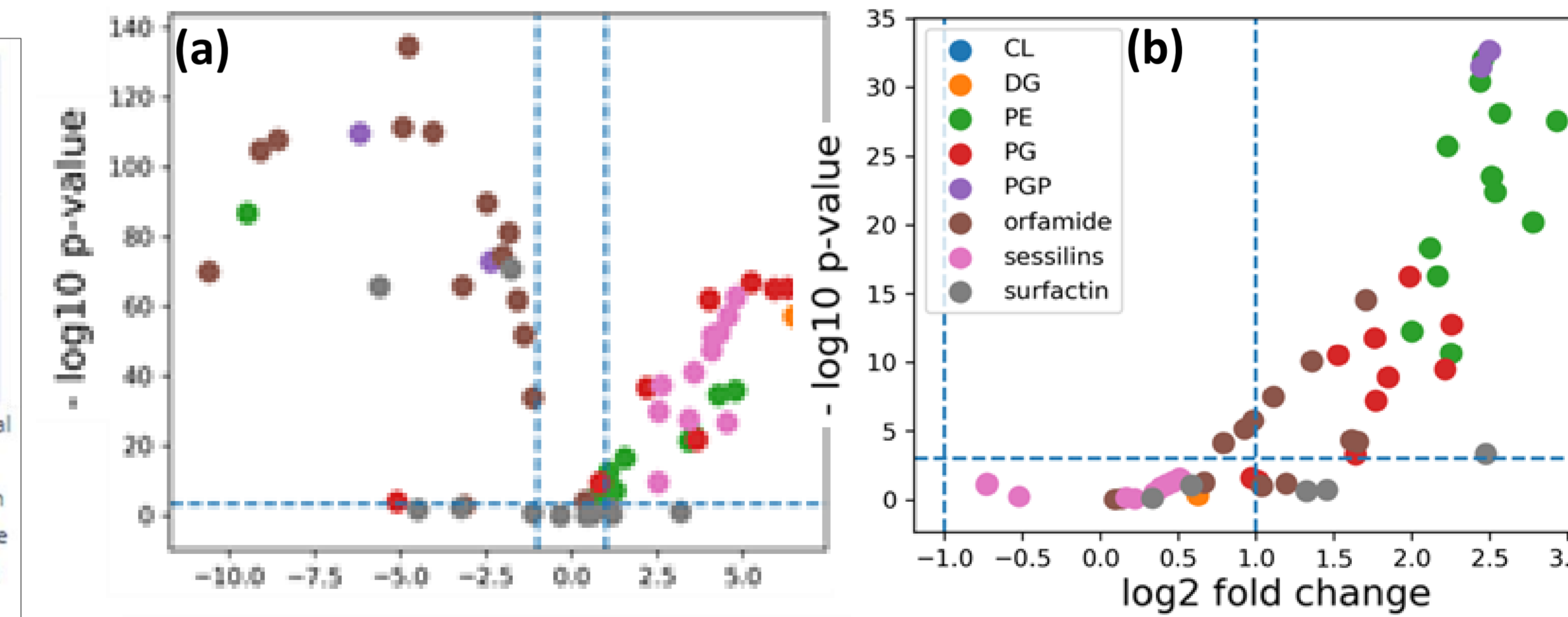
- (a) Signal comparison of *CMR12a* colonies against *GA1*.

- (b) To explore the signal heterogeneity of *CMR12a* in **interaction**, we performed a hierarchical clustering on the colonies and compared each cluster signals against the general population. One of those clusters contains colonies with a very distinct signals.

4. Conclusion

This new method allows the analysis and the detection of a very large number of bacterial colonies which helps to:

- Identify an over expression of lipids (PG) and sessilin of *CMR12a* colonies in contact with *GA1*.
- Identify outliers *CMR12a* colonies that over expressed (PE and PG) lipids and that under expressed sessilins.

[1] Rappez, L., Stadler, M., Triana, S. *et al.* SpaceM reveals metabolic states of single cells. *Nat Methods* **18,** 799–805 (2021).