Short Course : MS Imaging of bacteria and bacterial coculture

Pierre Burguet

Mass Spectrometry Laboratory, MolSYS RU, University of Liège

Summary

- Introduction : Mass spectrometry imaging in microbiology
- Example of TNA
- Method
- MIND project
- Testing of non-coated ITO slides
- Positive imaging
- Negative imaging
- Problem with MSI analysis
- Software of Dr Christopher Kune (c.kune@uliege.be)
- Example of peaks observed only in coculture

Introduction : Mass spectrometry imaging in microbiology

Imaging mass spectrometry tools allow the two-dimensional visualization of the distribution of trace metals, metabolites, surface lipids, peptides and proteins directly from biological samples without the need for chemical tagging or antibodies, and are becoming increasingly useful for microbiology applications such as fungi, bacteria or archea.

These method enable the generation of chemical hypotheses based on the spatial mapping of atoms and molecules that can correlate to or transcend observed phenotypes. The ability of IMS is to collect

molecular ‘snapshots’, at a certain time, of a given biological sample and superpose this molecular information onto an optical image.

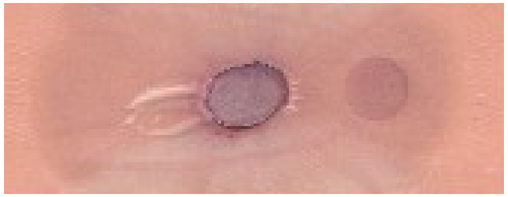
Example of TNA

Black spot disease of vanilla

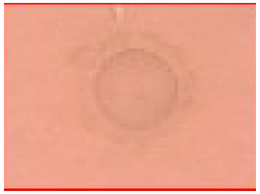
Anthracnoses are generally benign diseases that affect mainly the leaves, but sometimes also the stems and pods. They are caused by two species of fungi of the genus Colletotrichum which induce distinct symptoms. This disease develops in humid areas, on dense crops with little aeration. The disease is favored by wounds that can be caused by handling (pollination and punching in particular), or by biting insects. This disease is responsible for a loss of 10 to 50% of the production depending on the year. Translated with www.DeepL.com/Translator (free version)

Colletotrichum orchidophilum

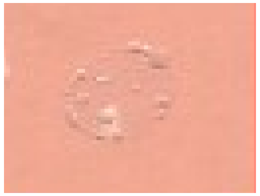
Interaction (bacillus 1)



Interaction (bacillus 2)



Bacillus 1



Bacillus 2

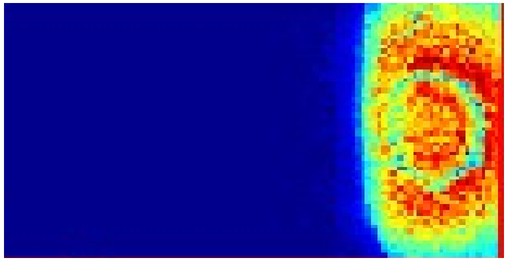
Example of TNA

Black spot disease of vanilla

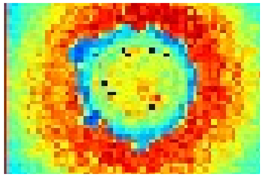
Anthracnoses are generally benign diseases that affect mainly the leaves, but sometimes also the stems and pods. They are caused by two species of fungi of the genus Colletotrichum which induce distinct symptoms. This disease develops in humid areas, on dense crops with little aeration. The disease is favored by wounds that can be caused by handling (pollination and punching in particular), or by biting insects. This disease is responsible for a loss of 10 to 50% of the production depending on the year. Translated with www.DeepL.com/Translator (free version)

Iturin

Colletotrichum orchidophilum

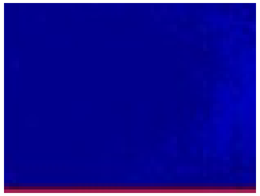


Interaction (bacillus 1)



Bacillus 1

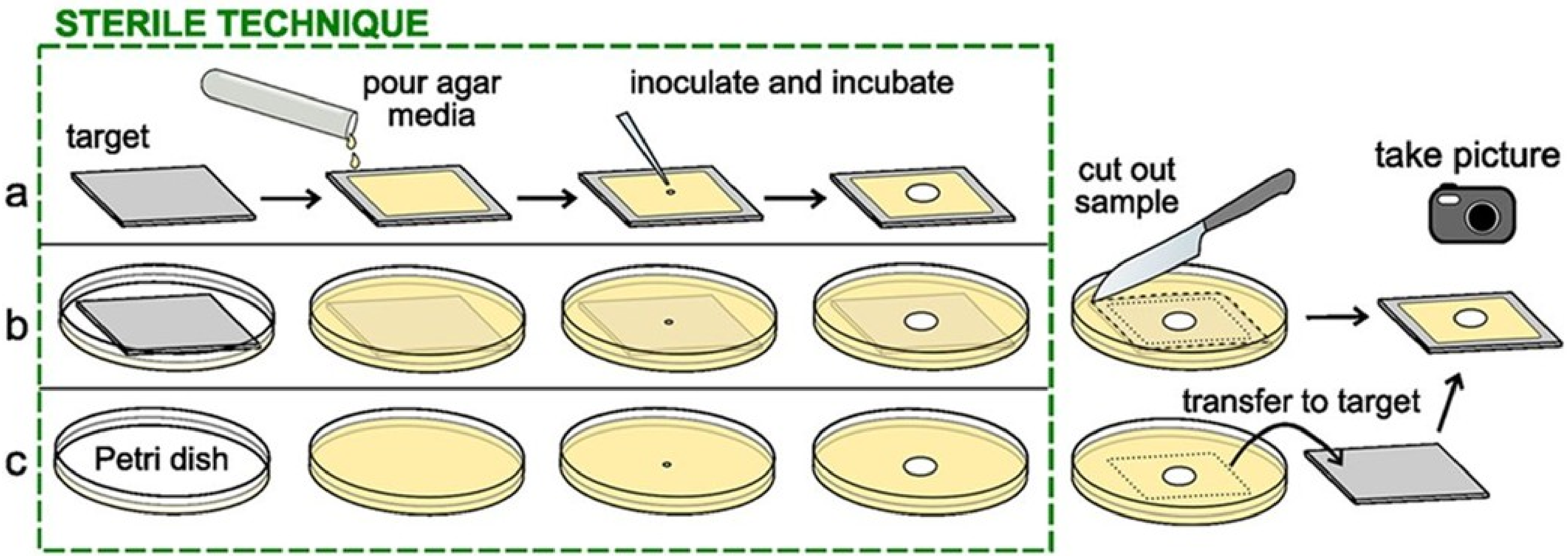
Interaction (bacillus 2)



Bacillus 2

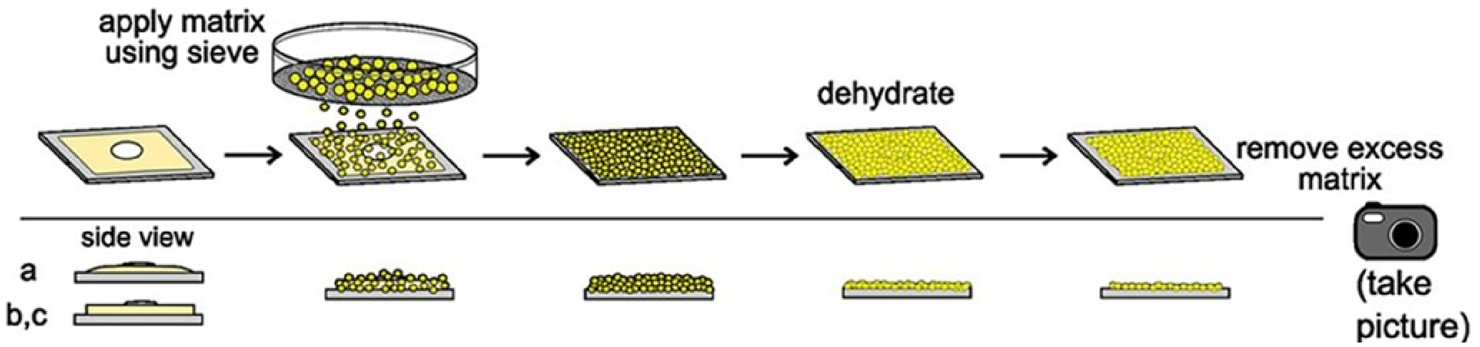
Method

1. Culture and transfer to lame :



Method

2. matrix application and dehydrataion :

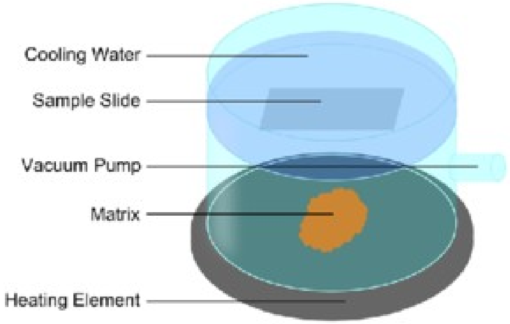


Different way of applying matrix :

a. Sieve :

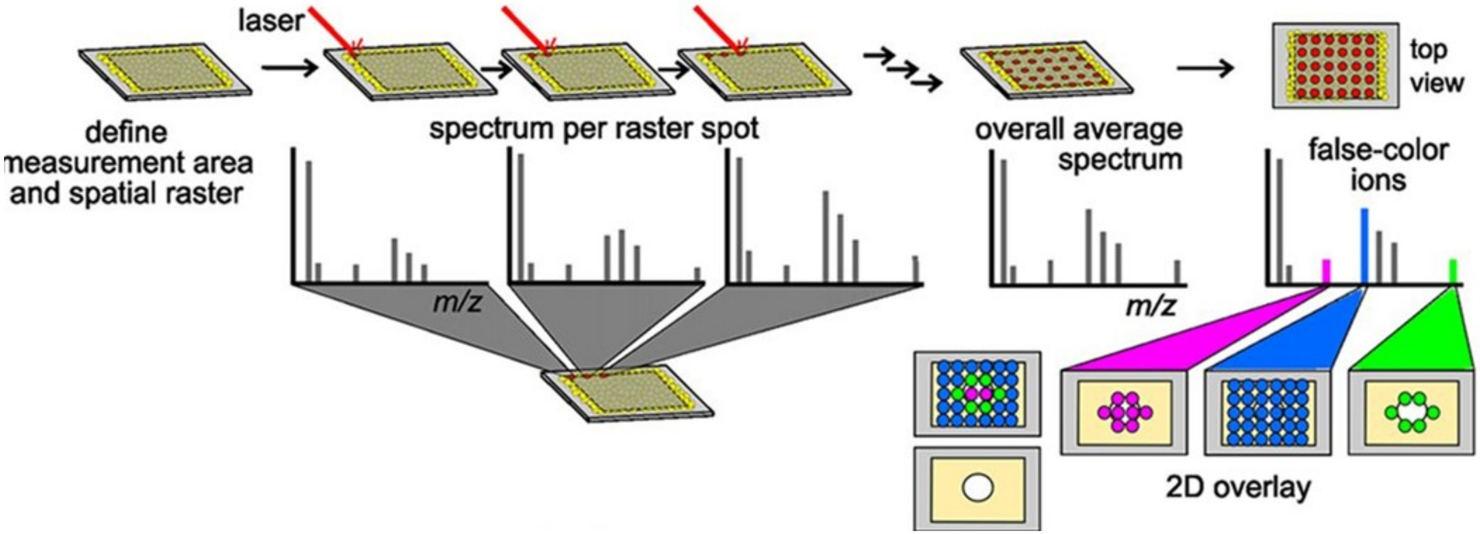
b. Spraying :

c. Sublimation



Method

3. MSI and Data analysis :



MIND project

MIND: **M**icrobial **I**nteractions for **N**atural Product **D**iscovery

Natural Product : A natural product is a chemical compound, generated by living organisms (such as plants, bacteria, fungi, insects,…) found in nature.

Microbial Natural products have now been used for decades in human and animal therapy (antibacterial, antifungals, antivirals, and anti cancer drug therapies) as well as in the agro industry (control plant diseases). Thus, they have largely participated in increasing the average life expectancy in developed countries

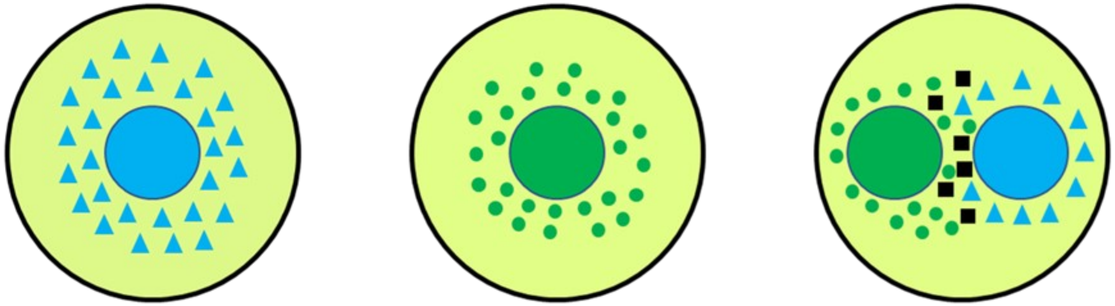
However, the range of new compounds released on the market in the last years is still rather low.

Microbial Interaction : Approach where two or more microorganisms are closely cultivated together. Co-culture can be an effective way to stimulate the production of substances that are not formed during pure culture. These co-culture approaches attempt to mimic the natural ecological situations where these microbes coexist and may therefore be able to express many biosynthetic gene clusters (BCGs) involved in the synthesis of still unknown metabolites.

MIND project

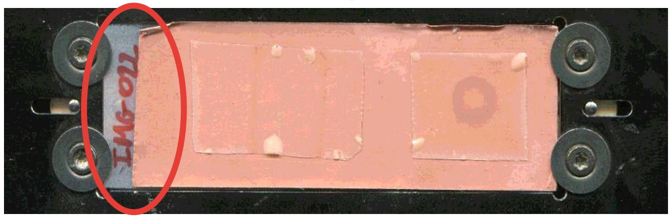
MIND: **M**icrobial **I**nteractions for **N**atural Product **D**iscovery

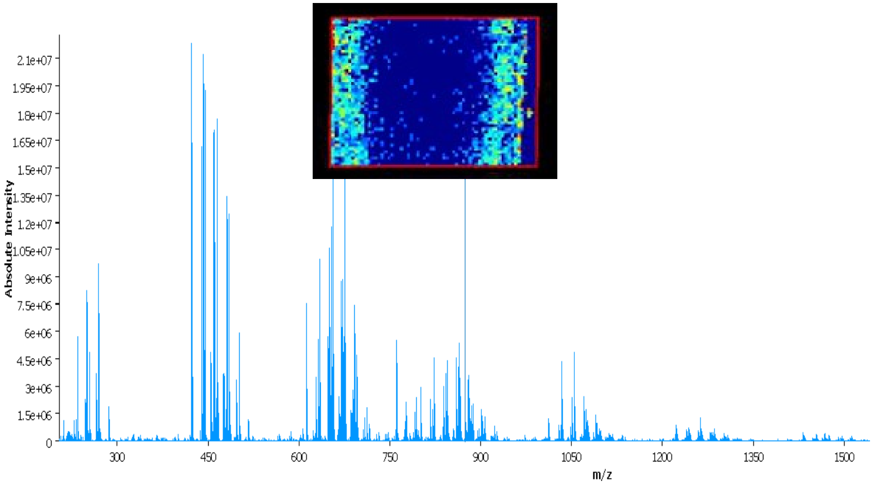
Bacterial Co-culture : For the project, we decided to work with two ecologically ubiquitous species among the most prolific bacteria regarding the potential to form highly active BSM, Bacillus and Streptomyces. These bacteria produce a wide range of chemically diverse products such as terpenes, non-ribosomal (lipo)peptides, polyketides, ribosomally synthesized and post-translationally modified peptides (RiPP), encoded by well identified Biosynthetic Gene Clusters (BGCs). Moreover, genomics, which is constantly growing, highlights the exceptional richness of these bacteria, which possess within their genomes a large number of cryptic BCGs coding for unknown BSMs.



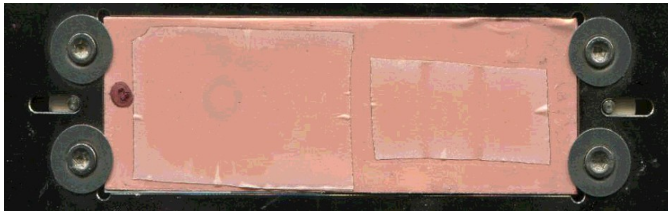
Testing of non-coated ITO slides

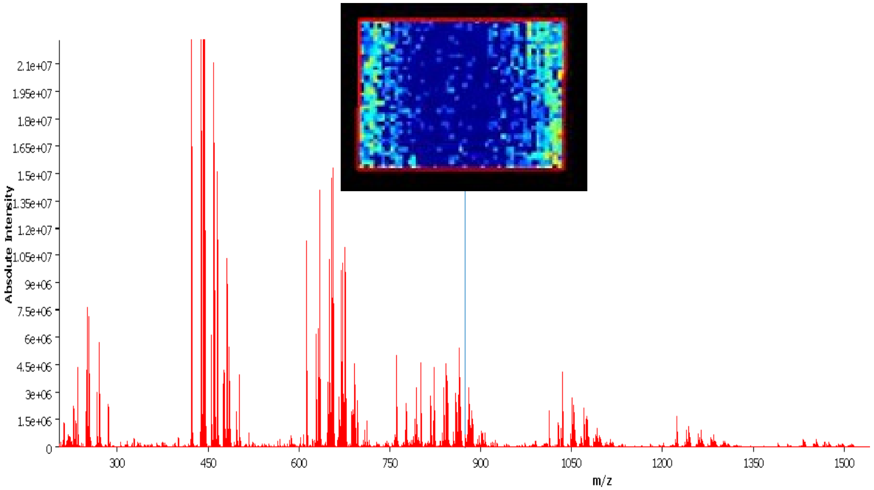
ITO slide
(>10€/pièce)





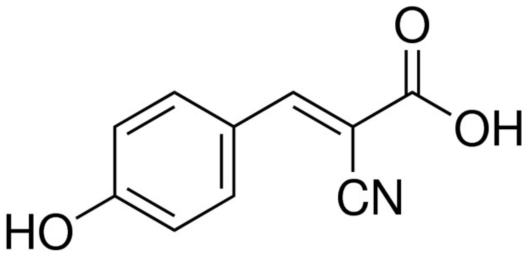
non-ITO slide
(≈0,05€/pièce)





For 100 images ➔ gain of 1500 €

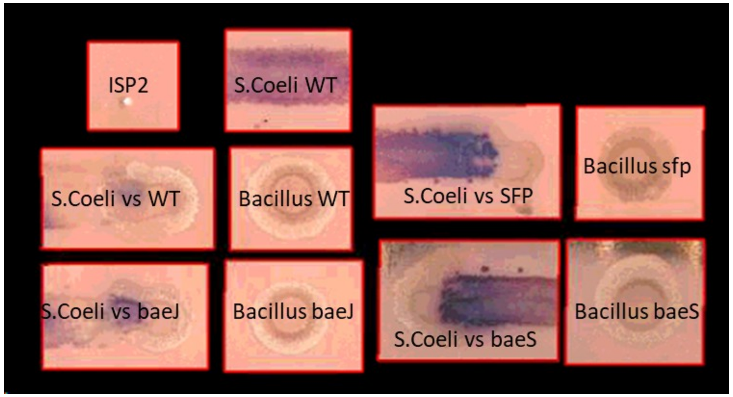
Positive imaging

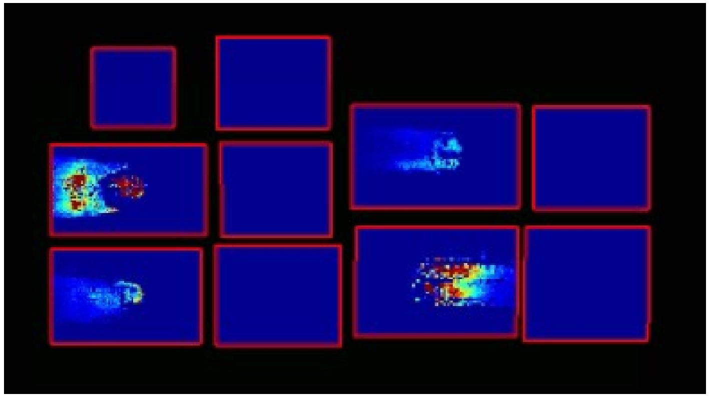


→ Allows positive ionization
Note : HCCA is the most commonly used matrix in imaging

α-Cyano-4-hydroxycinnamic acid (HCCA or CHCA)

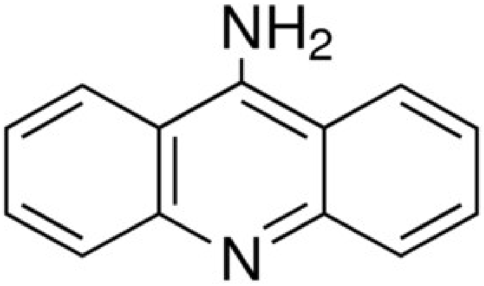
Streptomyces coelicolor – Bacillus GA1 (WT, BaeJ, BaeS and sfp)





Metabolite
367.1813 (m/z)

Negative imaging

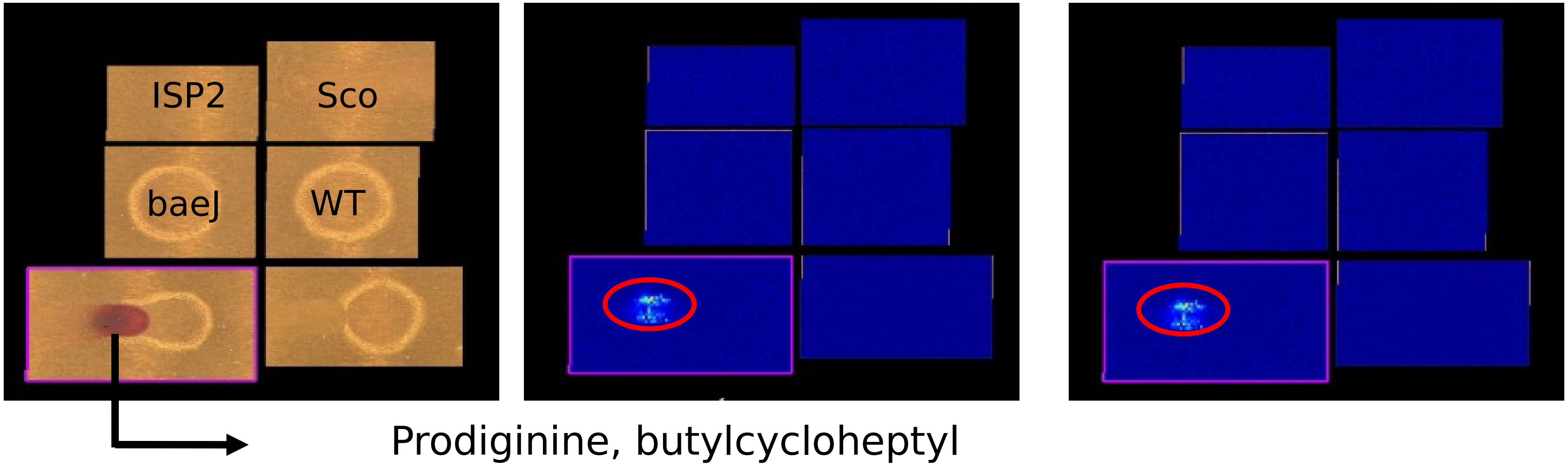


9- aminoacridine

→ Allows negative ionization ("*weak base*")
Note : method (MALDI -) low using occurrence in MALDI imaging

Streptomyces coelicolor – Bacillus GA1 (WT and BaeJ)

658.148 660.14 (m/z)

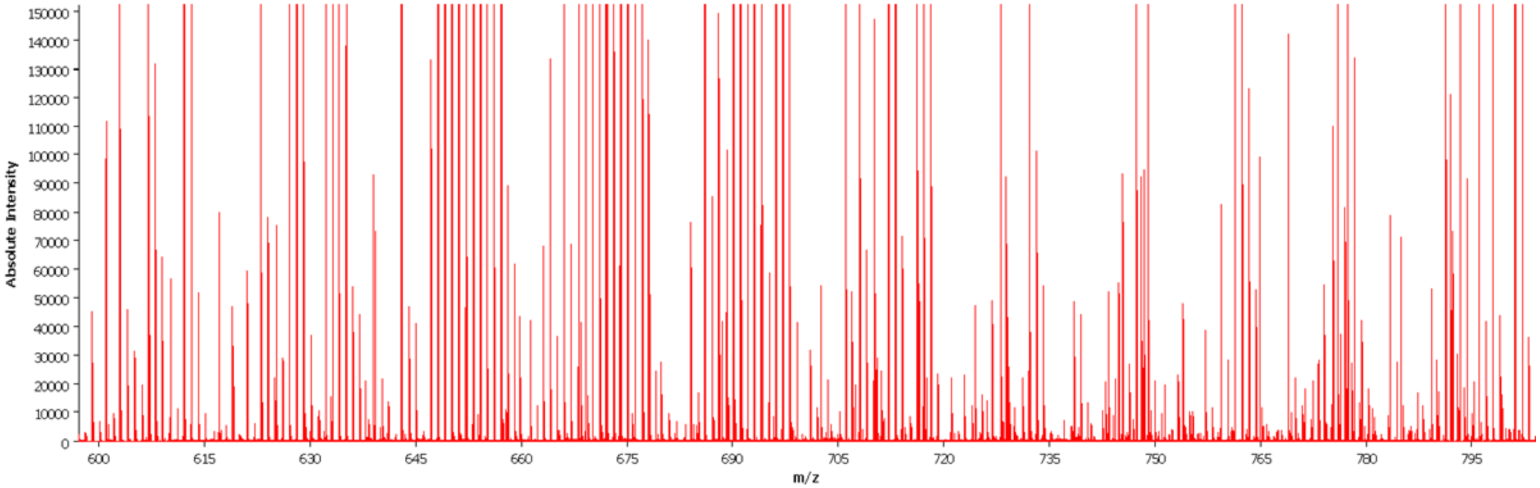


Prodiginine, butylcycloheptyl

Note : Prodiginines has anticancer and immunosuppressive properties

Problem with MSI analysis

The current analysis of spectra, even pseudo computerized, is extremely long, tedious task, and delicate.
→ On average, there are several thousand peaks per image.

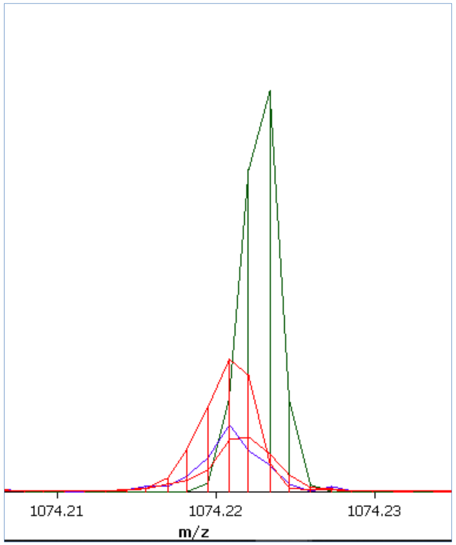


➔ How to easily identify signals corresponding to metabolites ?

Software of Christopher

The data analysis could be optimized by an in-house software that would compare the spectra of interest: Ex: Bacillus vs [Bacillus + Strepto.]

→ Basis for reflection: The signals observed for several different MSI runs overlap almost perfectly

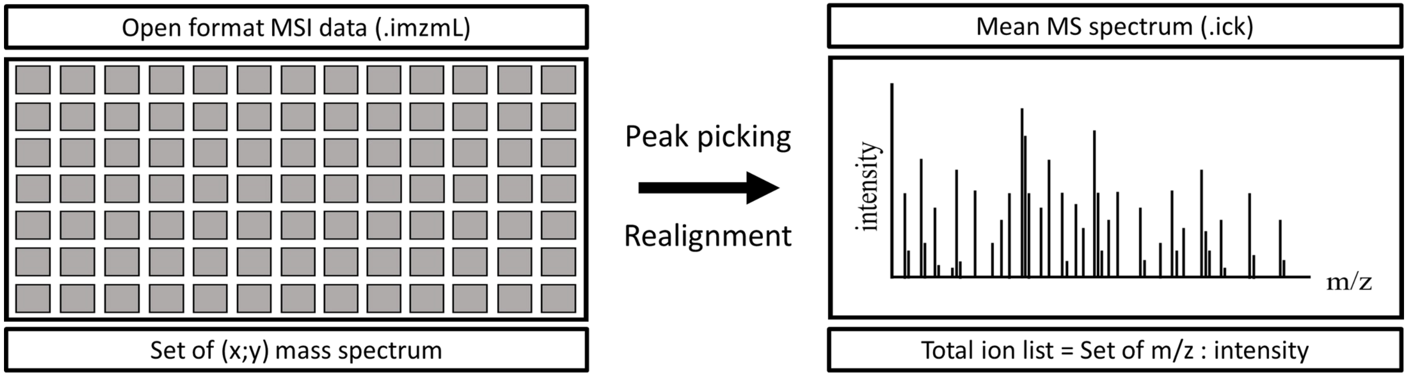


Legend :
- Interaction
- Bacillus
- Streptomyces

➔ We developed a Python script that help us to find more easily the specific peaks in one of the image vs ref. (collab. Dr. Christopher Kune)

Software of Christopher

1. Pre-processing of each image sample.


Open format MSI data (.imzmL)

Set of (x;y) mass spectrum

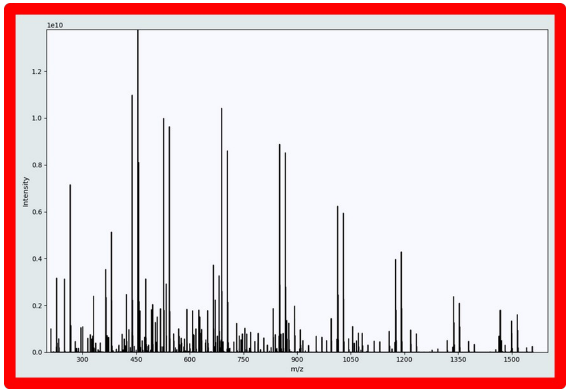
Peak picking

Realignment

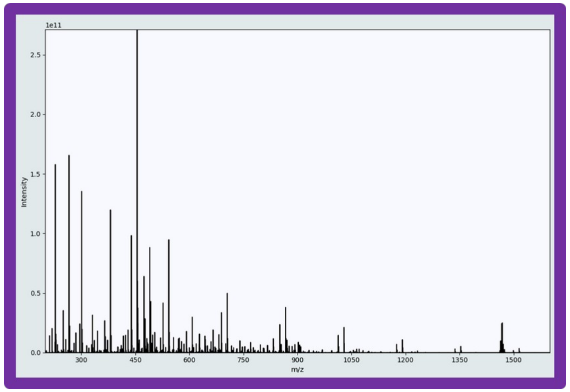
Mean MS spectrum (.ick)

Total ion list = Set of m/z : intensity

→ this step is repeated for each condition



culture medium (ISP2) + matrix (+)

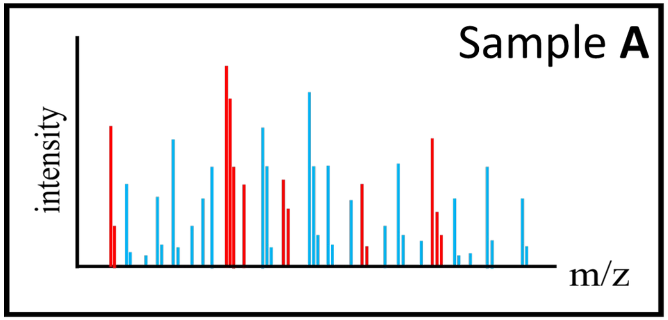


Escherichia coli (+)

Software of Christopher

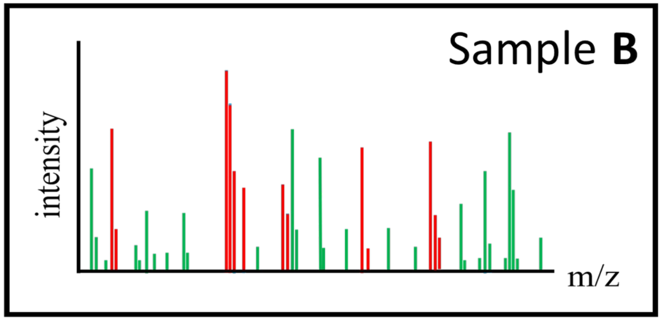
2. Comparison of two samples A and B

Signal specific to A / Signal specific to B / Common signal to A and B

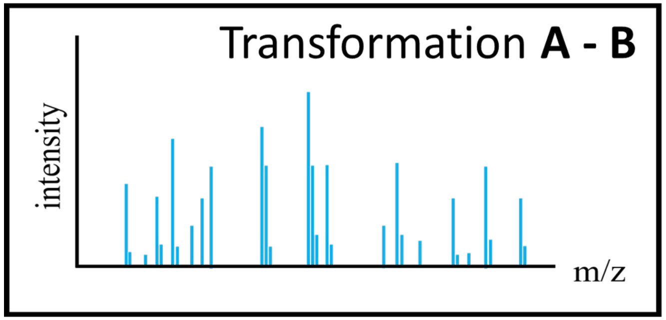


Total ion list of sample **A**

Comparison



Total ion list of sample **B**



Total ion list of sample A – sample B

Visualization

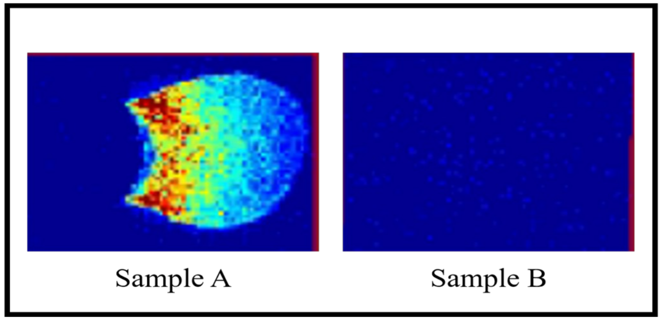
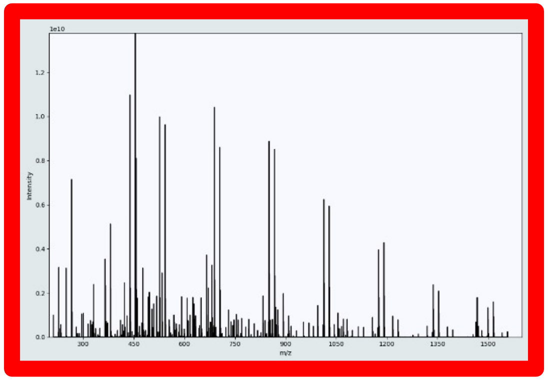


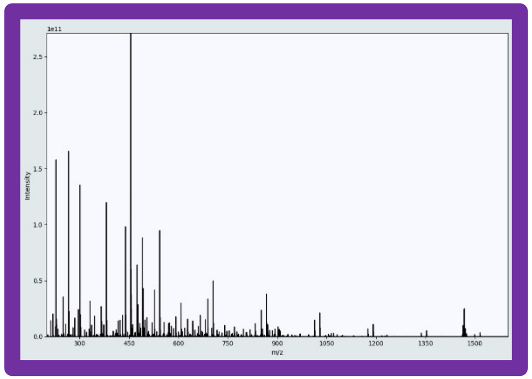
Image generated from one of the conserved ions

Software of Christopher

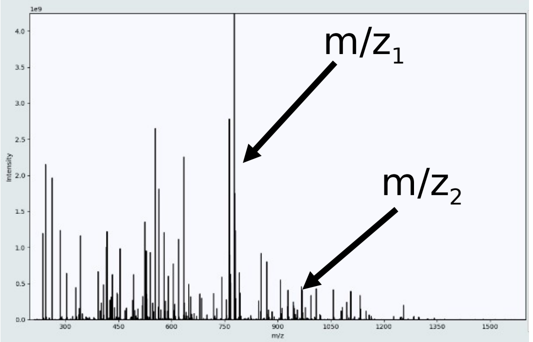
Example of Transformation: “Metabolome”



ISP2



Escherichia coli



Escherichia coli – ISP2

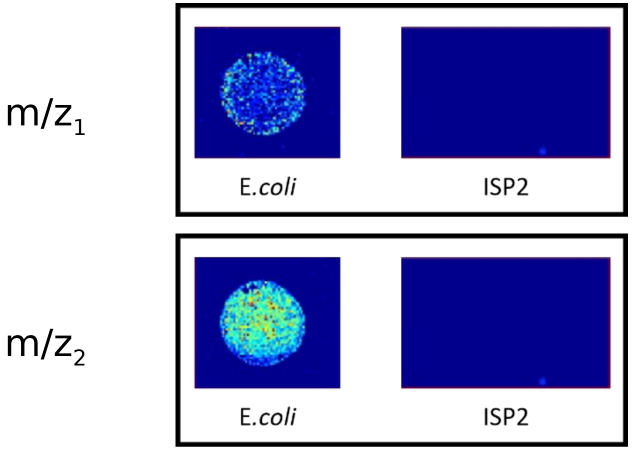
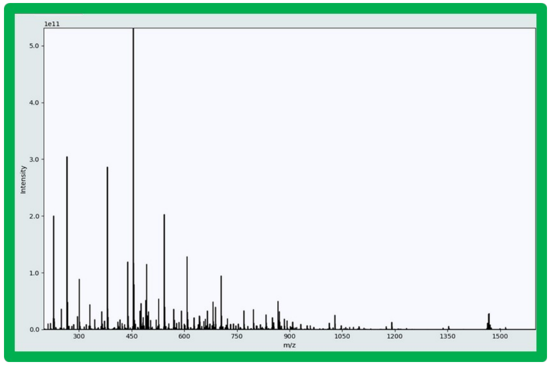


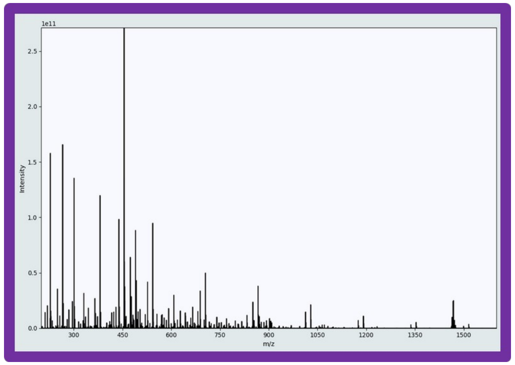
Image generated from one of the shared m/z

Software of Christopher

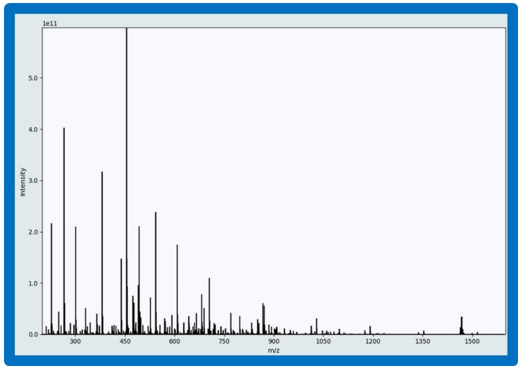
Example of Transformation: “Interactome”



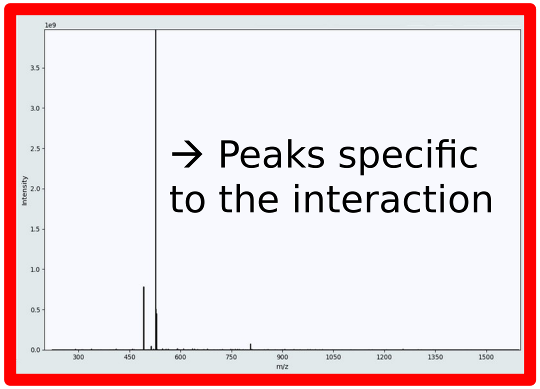
Streptomyces coelicolor



Escherichia coli

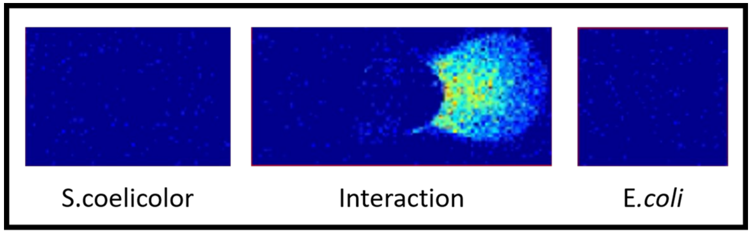


Interaction



→ Peaks specific to the interaction

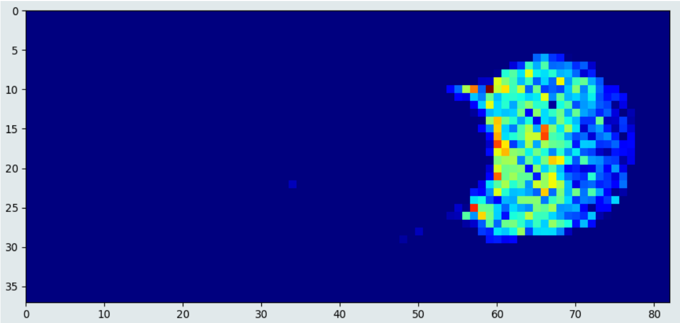
Interaction – S. coelicolor – E.coli

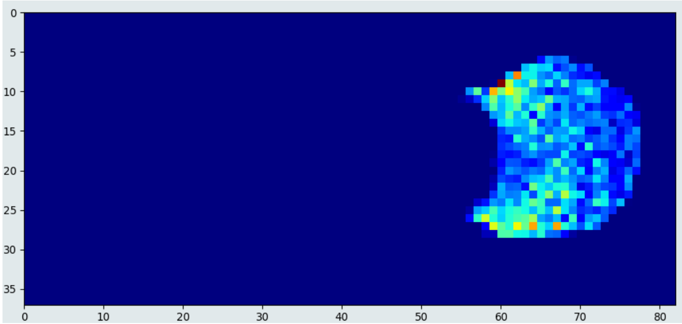


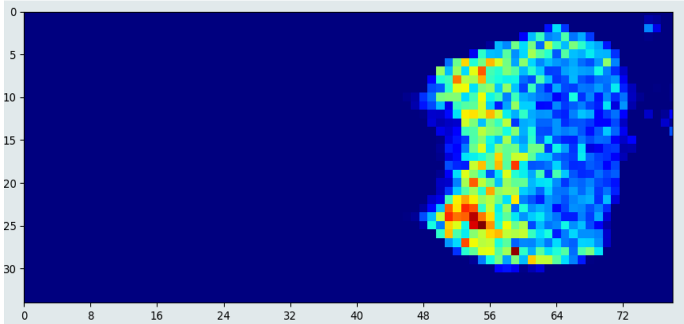
S.coelicolor Interaction E.*coli*

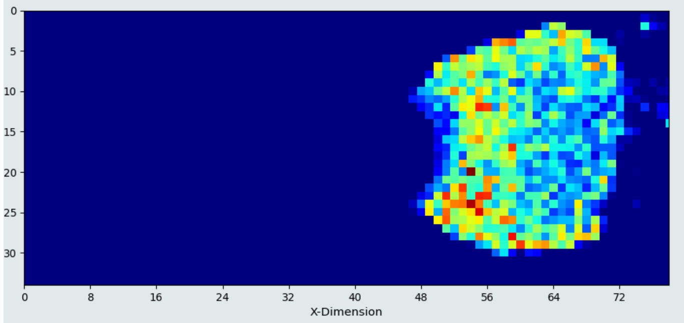
Image generated from one of the shared m/z

Example of peaks observed only in co-culture


m/z 492.25 (+)


m/z 527.38 (+)


m/z 647.34 (-)


m/z 453.28 (-)

Note : Positive and negative imaging gives complementary information