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University of Strathclyde Glasgow

Metabolomics and Dereplication Strategies in Natural Products Discovery

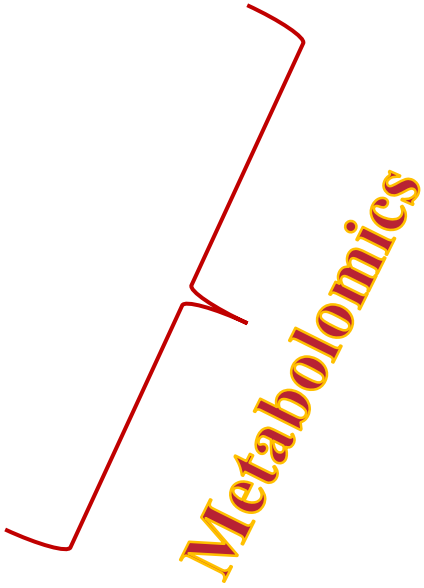
by

Ahmed Tawfike

Introduction

- **Challenges with NP discovery**
  - › Long process separation → time consuming
  - › Redundant isolation of known compounds
  - › Interactions
  - › Structure elucidation
- **Solutions**

  Rapid dereplication (metabolites annotation)
  - › NP libraries e.g. DNP, AntiBase or MarineLit
  - › Bioassay guided fractionation
  - › Improvement of separation
  - › LC-HRMS, MS-MS and NMR
  - › Differential analysis software for data mining
  - › MVDA software

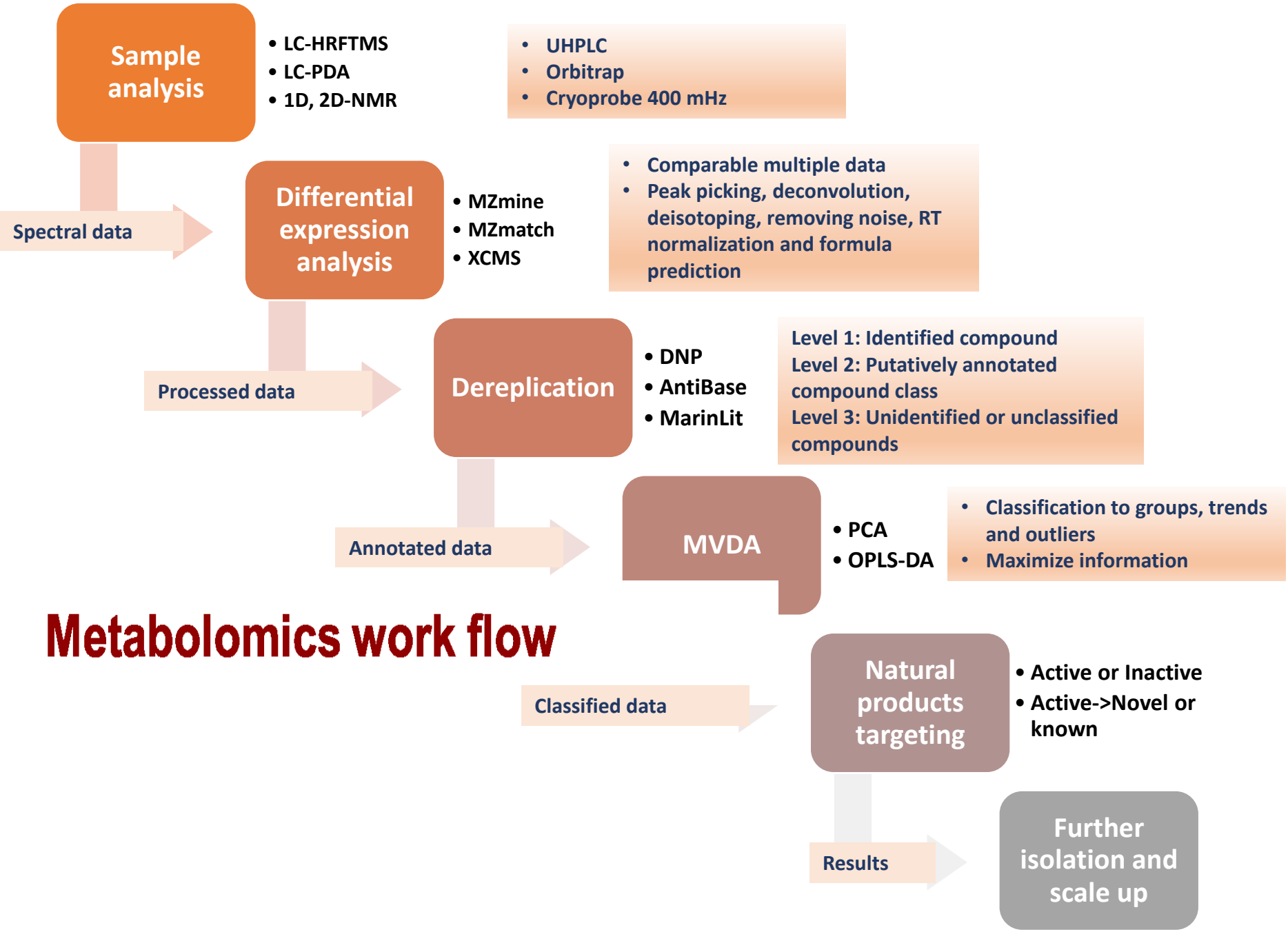


Definitions

- **Metabolome:** The complete set of small molecules found in a cell, tissue or organism at a certain point in time
- **Metabolomics:** Comprehensive qualitative and quantitative analysis of all metabolites contained in an organism, tissue or cell at a specific time and under specific condition
  - **Metabolites Fingerprinting:** Rapid classifications of samples. The purpose is not identify each individual metabolite but to compare patterns or fingerprints of metabolites. Untargeted
  - **Metabolites Profiling:** Extensive identification and quantitation of metabolites either related to a specific pathway or class of compounds. More targeted

Dereplication: Testing sample mixtures that are active in screening in order to differentiate the novel compounds from the active substances that have already been studied

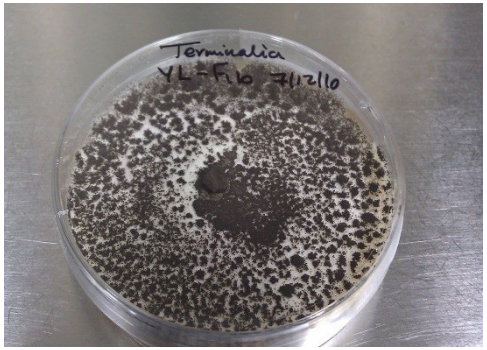
  - **Metabolites Target Analysis:** Investigation of metabolites that are related to a specific pathway to observe the specific metabolic modifications that may be related to a particular change e.g. genetic modification


Sample analysis
• LC-HRFTMS
• LC-PDA
• 1D, 2D-NMR
• UHPLC
• Orbitrap
• Cryoprobe 400 mHz
Spectral data
Differential expression analysis
• MZmine
• MZmatch
• XCMS
• Comparable multiple data
• Peak picking, deconvolution, deisotoping, removing noise, RT normalization and formula prediction
Processed data
Dereplication
• DNP
• AntiBase
• MarinLit
Level 1: Identified compound
Level 2: Putatively annotated compound class
Level 3: Unidentified or unclassified compounds
Annotated data
MVDA
• PCA
• OPLS-DA
• Classification to groups, trends and outliers
• Maximize information
Metabolomics work flow
Classified data
Natural products targeting
• Active or Inactive
• Active->Novel or known
Results
Further isolation and scale up

Example

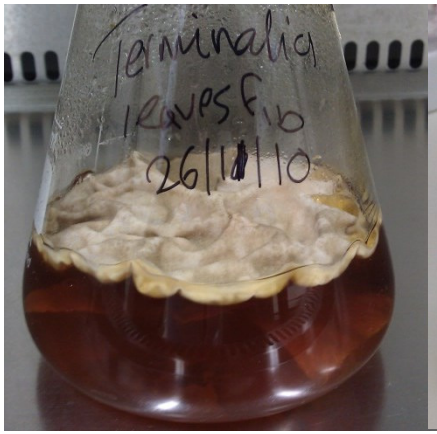
Fungal endophyte from *Terminalia laxiflora*

***Terminalia laxiflora* leaves**
Combretaceae

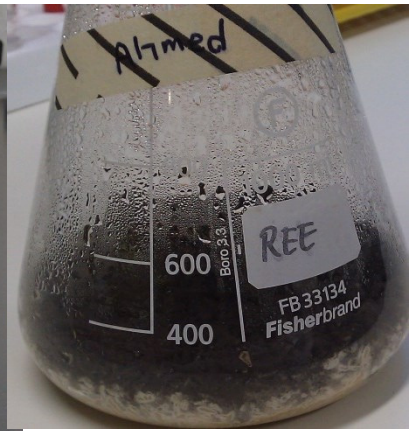


Aspergillus aculeatus
on MA plate

Uses:
Bark Medicines:
yaws
Leaf, root Medicines:
diarrhoea, dysentery
Plant Medicines:
pulmonary troubles



Liquid culture in
Wickerham medium

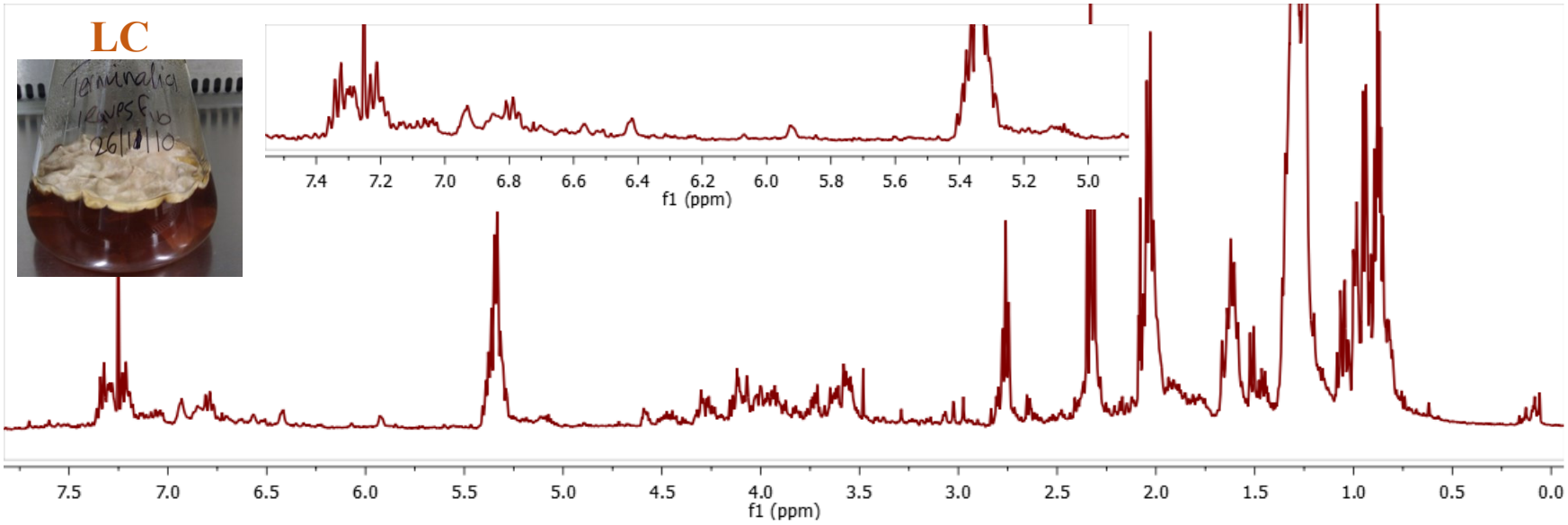


Solid rice culture

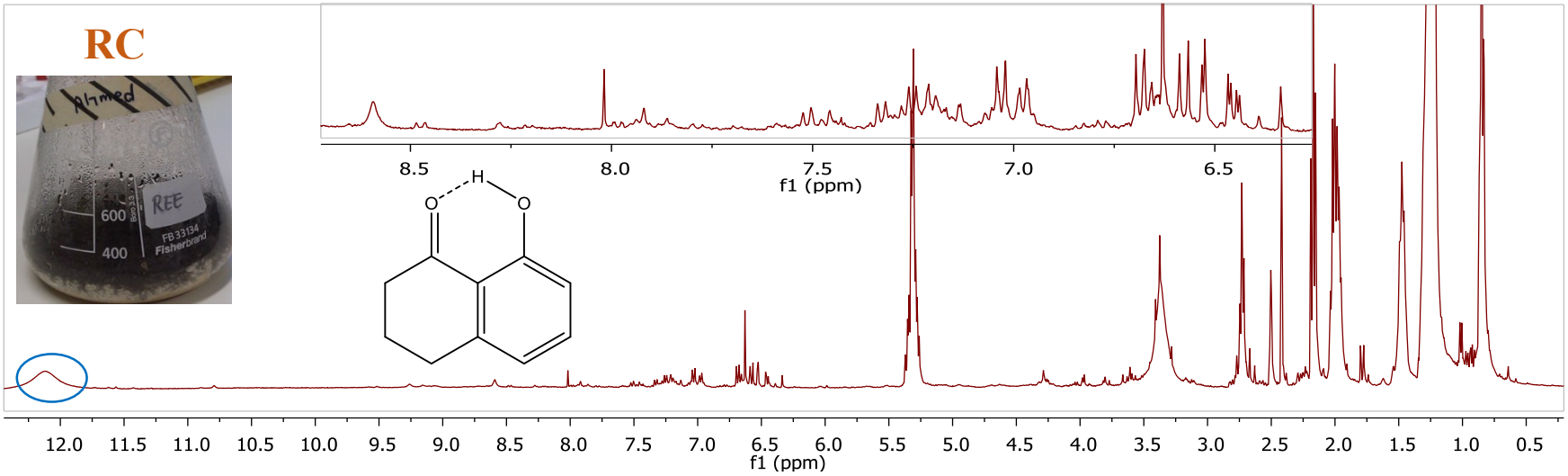
Metabolites fingerprinting of different culture extracts

Dereplication study by NMR spectroscopy

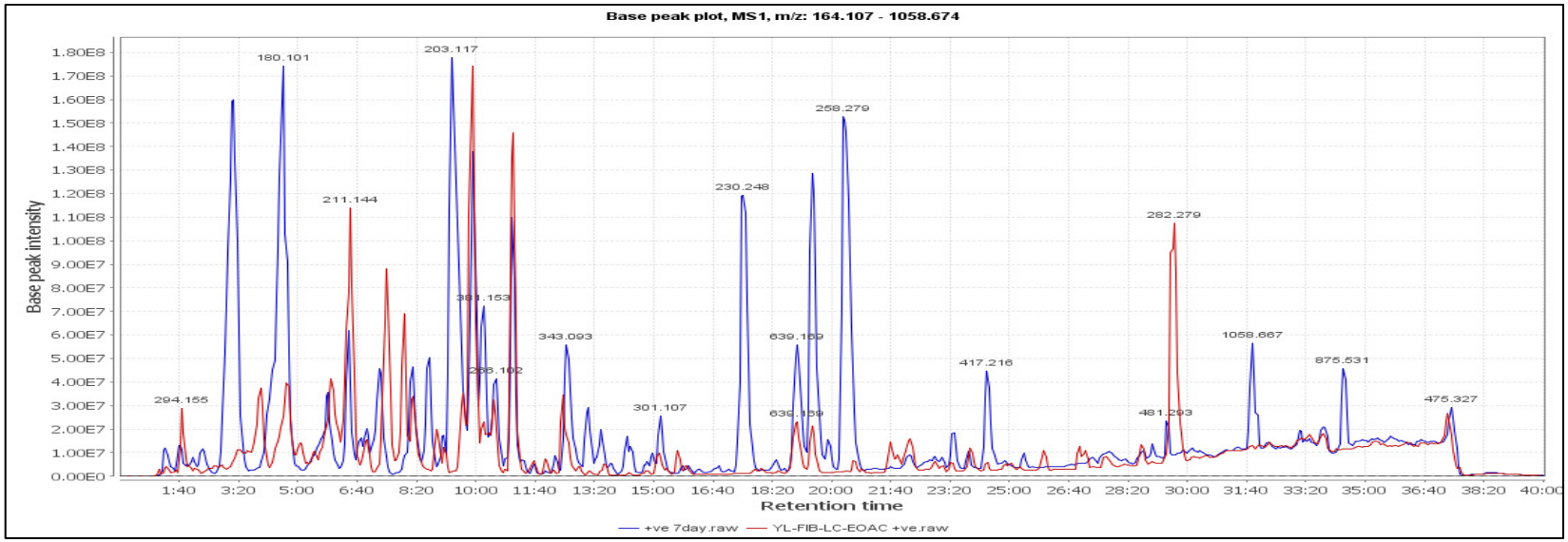
LC



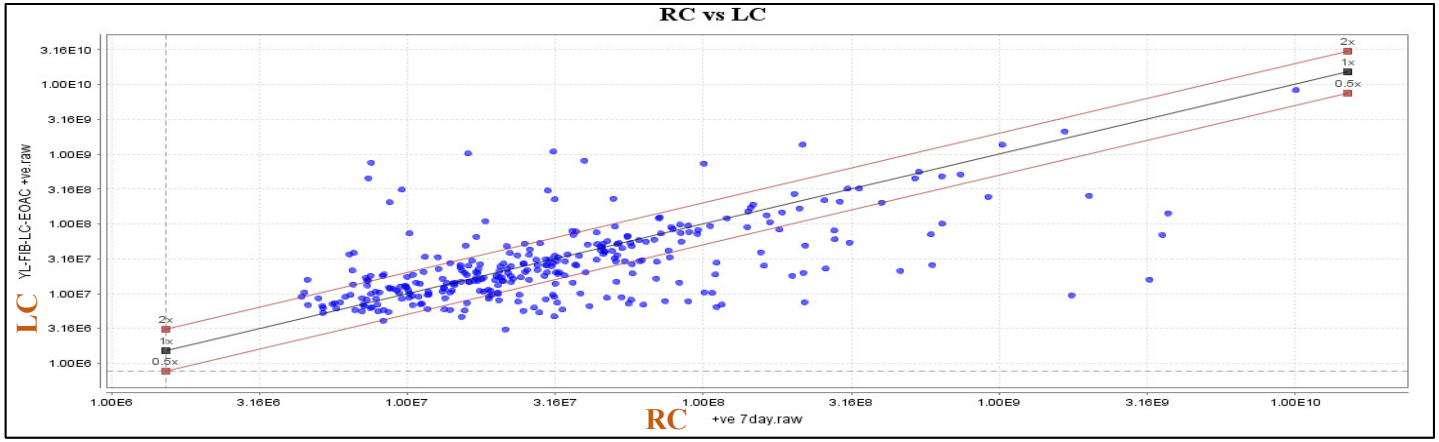
RC



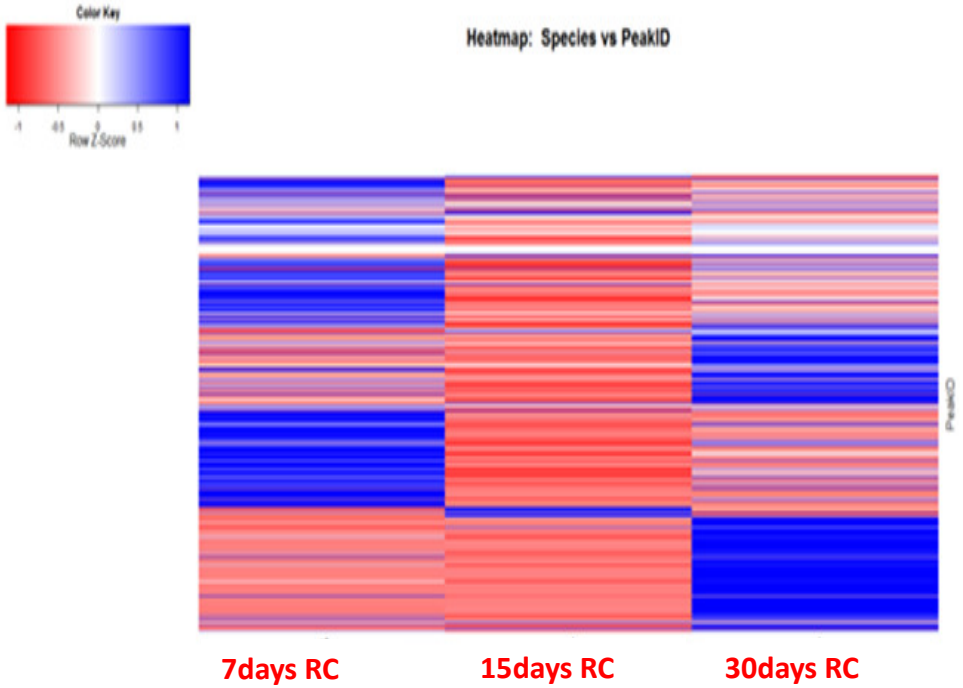
Metabolites profiling using HR-MS by Mzmine



● RC ● LC



Metabolites fingerprinting using R software



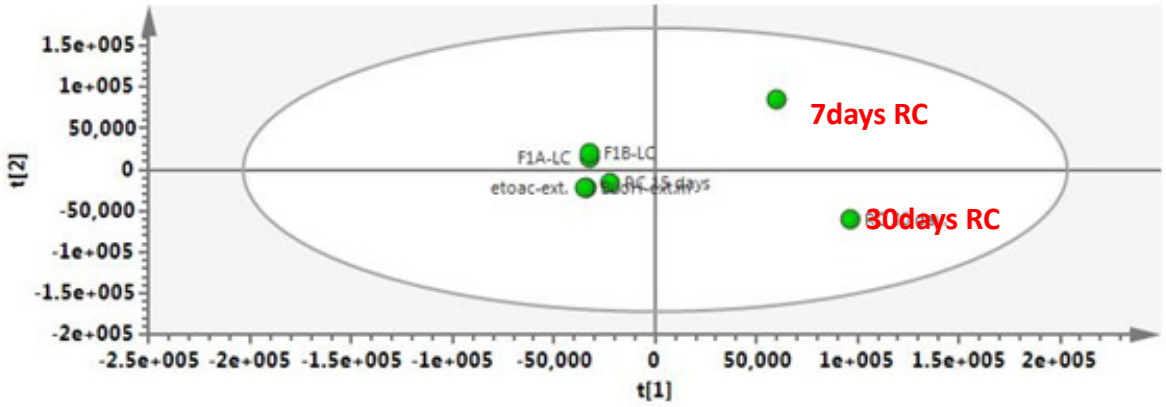
Heat Map for different rice culture extracts

Dereplication Table

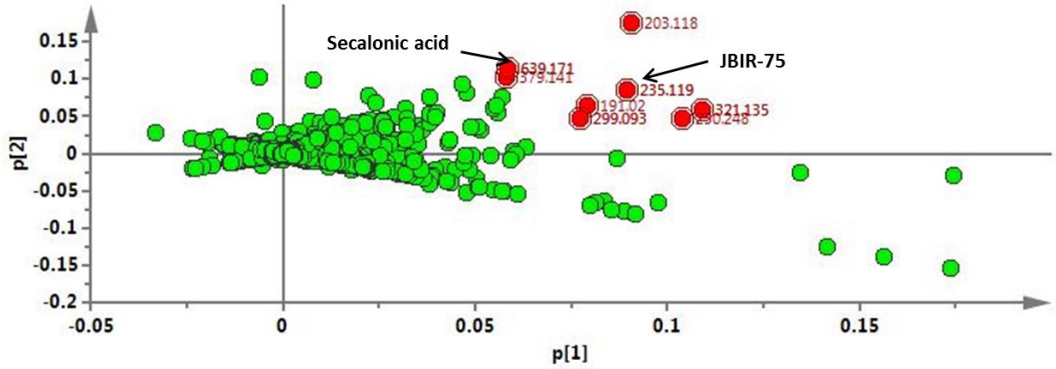
row m/z	row RT	Formula/ Name	LC	BuOH plant ext.	EtOAC plant ext.	RC-7days	RC-15days	RC-30days
203.118	9.39	$C_{12}H_{14}N_2O$ Nb-Acetyl-tryptamine	1.89E+07	0	0	2.97E+09	7.66E+06	4.89E+07
180.102	4.33	$C_{10}H_{13}NO_2$ Phenyl-alanine-N-Me	1.37E+08	9.83E+03	5.45E+03	2.05E+09	6.27E+06	6.83E+07
235.119	3.21	$C_{11}H_{14}N_4O_2$ JBIR-75 *Aspergillus sp.* fS14	7.11E+07	0	0	1.93E+09	3.03E+07	6.75E+08
475.326	36.46	$C_{25}H_{46}O_8$ Unknown	2.31E+09	7.49E+02	0	1.76E+09	1.78E+09	1.77E+08
639.171	19.22	$C_{32}H_{30}O_{14}$ Secalonic acid D *A. aculeatus*	6.28E+08	0	0	1.57E+09	4.88E+07	1.48E+08
436.198	9.95	$C_{22}H_{29}NO_8$ Neoxaline *A. japonicus*	2.28E+09	0	6.96E+02	1.05E+09	9.49E+07	5.13E+08
340.259	8.19		5.05E+08	0	0	8.75E+08	1.18E+09	9.86E+08
381.155	9.57	$C_{19}H_{24}O_8$ Dinaphtho[2,1-b:1',2'-d] furan-5,9-dione *Sphaeropsidales* sp.	1.95E+05	2.85E+02	1.96E+03	7.24E+08	3.48E+06	4.96E+07
343.092	12.46		2.49E+08	0	0	7.02E+08	3.30E+07	3.45E+08
478.27	11.01	$C_{28}H_{35}N_3O_4$ ParaherquamideE *A. aculeatus*	1.38E+09	0	0	6.34E+08	5.22E+07	1.67E+08
405.229	9.00	$C_{23}H_{32}O_6$ Phalarine *Pharalis coerulescens*	2.45E+04	4.61E+03	2.02E+03	5.83E+08	3.49E+04	3.17E+05

Principle Component Analysis (PCA)

Score plot

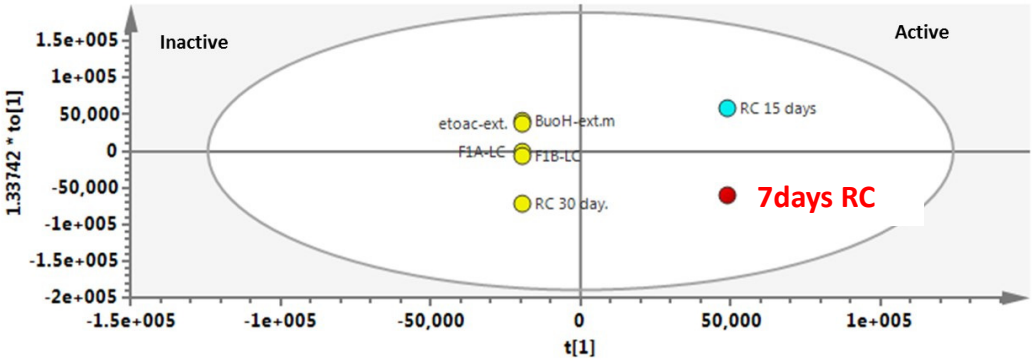


Loading plot



Supervised Analysis (OPLS-DA)

Score plot



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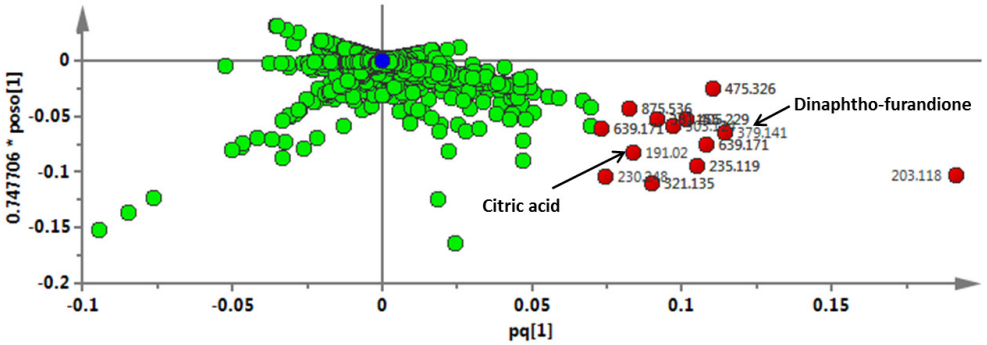
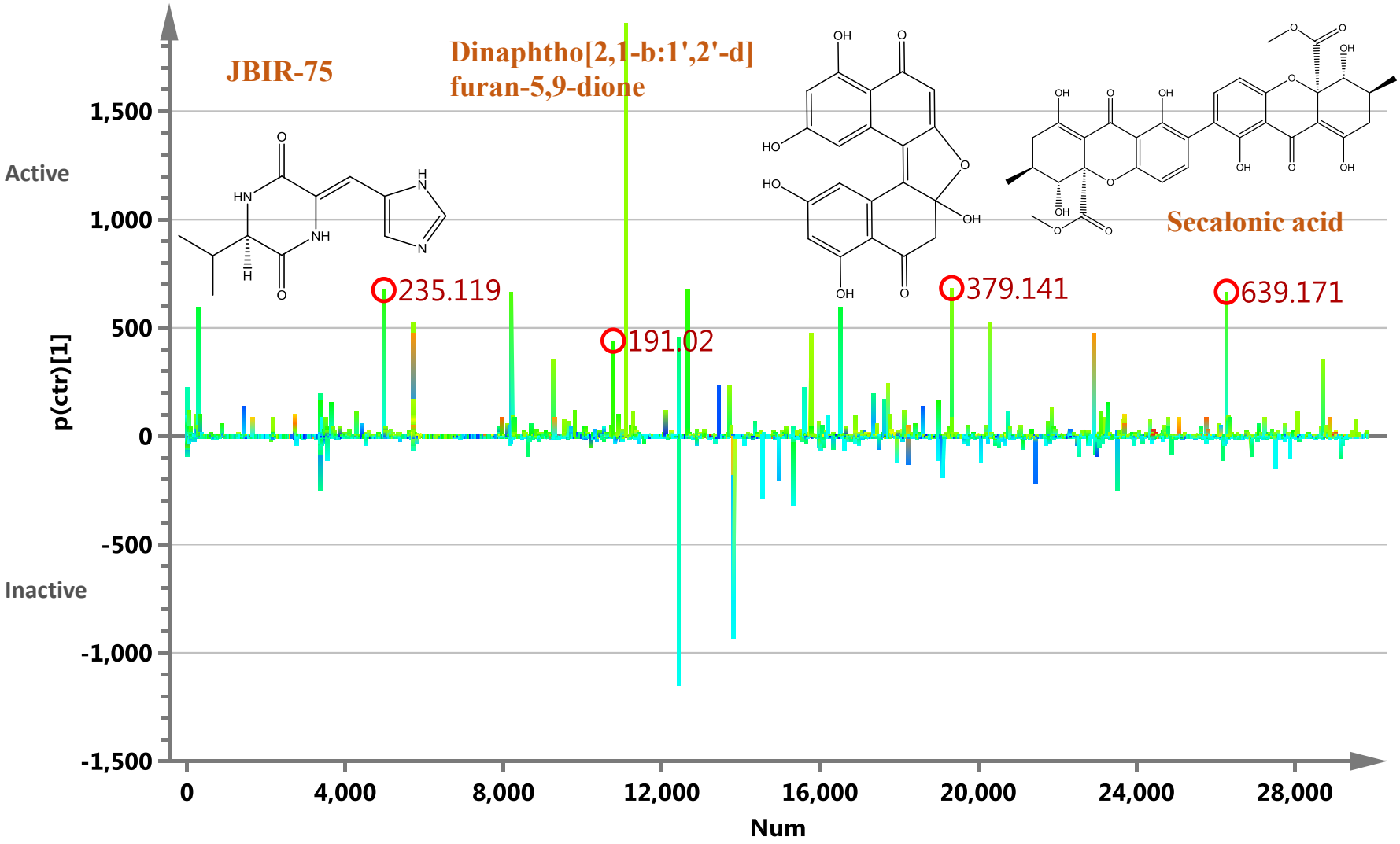


Isolated compounds on S-line



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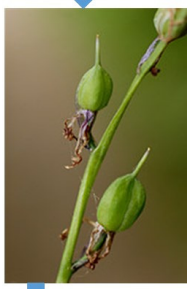
High Value Chemicals from Plants

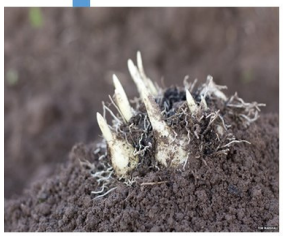
BEACON
From plants to products
O blanhigion i gynhyrchion

British Bluebell

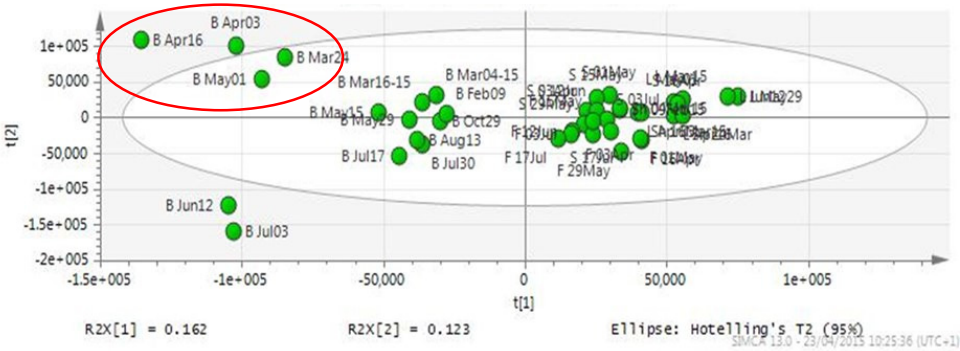
May - June



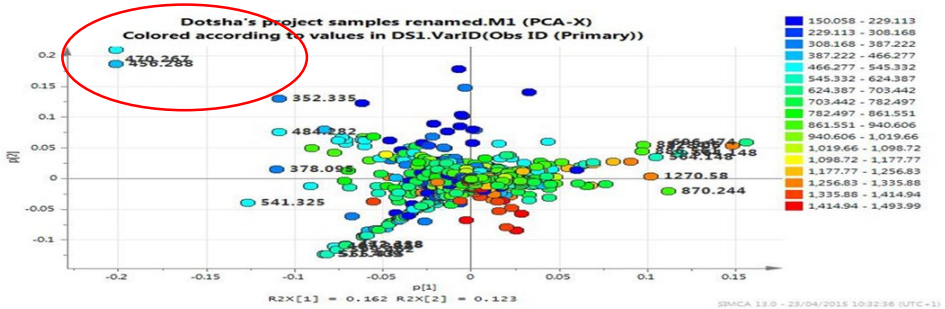
August



September - January



PCA score plot



PCA loading plot

SEVENTH FRAMEWORK PROGRAMME

SEABIOTECH

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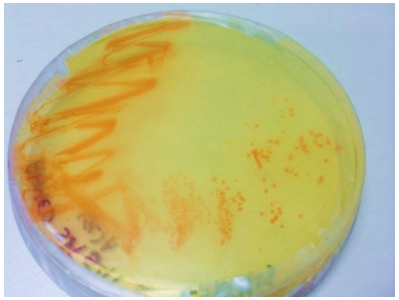

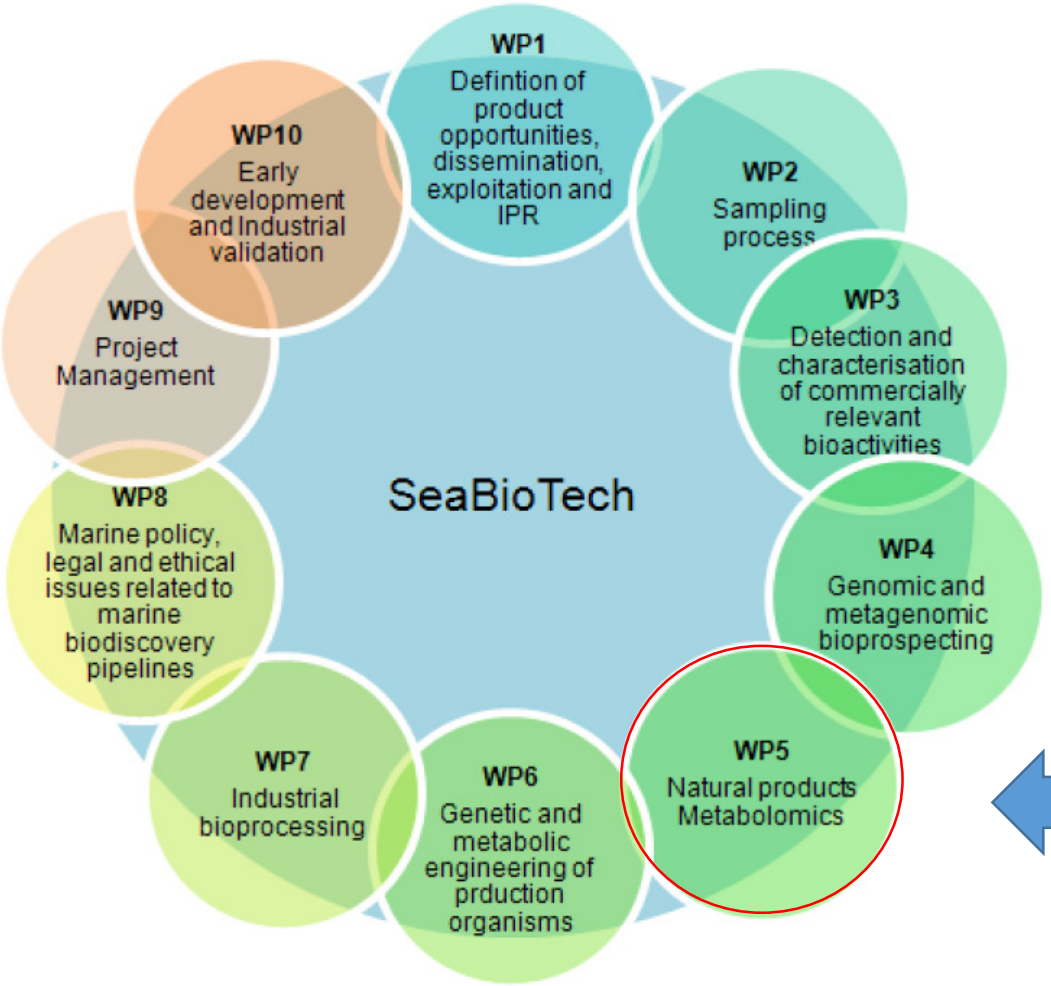

From sea-bed to test-bed: harvesting the potential of marine biodiversity for industrial biotechnology

Conclusion

Applications of metabolomics

- Improve identification and dereplication steps
  - Optimization of fermentation and cultivation media
  - Early prediction of the active compounds
  - Prioritizing the fractions subjected to fractionation
  - Metabolomics can be used to detect biomarkers and/or precursors to dereplicate the biosynthesis of the natural product at different development stages of their biological source as well as simultaneously screen for the bioactivity
  - Highlighting seasonal variation in the produced secondary metabolites
  - Link chemical profile and bioactivity pattern of phytomedicines

- Quality control of phytomedicine
- Proof of efficacy and mode of action
- Bioavailability and toxicity assessment of NP

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