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COMMENTARY

High Resolution-Mass Spectrometry as a unique Bioanalytical Tool in Natural Product Studies

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Natural products (NP), especially, bacterial secondary metabolites, still represent a gorgeous font of chemical compounds for drug discovery. Advances in genome sequencing and mining, in addition to bio-synthetic pathway manipulation, allow the expression of silent (cryptic) gene clusters and provide the access to previously underexplored sources such as new groups of microorganisms, consequently highlighting that is possible to discover new chemical entities. A fundamental role is played by the progress in compound detection technologies. Indeed, the main intent is to shorten the time necessary to discard known compounds and identify the new ones. High resolution mass spectrometry (HRMS) has become a reliable detection responding to several analytical challenges. Actually, the employment of Mass Spectrometry has grown up like a giant tree developing many branches and a thick crown that invaded almost all research fields regarding the omics sciences (metabolomics, proteomics, lipidomics, etc.) [1]. Hence, an extraordinary time started to strongly stimulate analytical data acquisition; it became possible to directly analyze crude extracts without the need for purification/ isolation of specific species and large data sets can be processed at once [2]. Several experimental databases, such as Dictionary of Natural Products DNP, ChemSpider, REAXYS, and several software tools such as METLIN, MIDAS and MetFRAg [3] can be used for metabolite identification in metabolomics; molecular structures were provided and matching of measured mass spectra (MS/MS) against the predicted fragments of metabolites can be performed although very often the signals found do not correspond with the described ones [4]. MS based metabolomics became the technique of choice for rapid detection and dereplication of secondary metabolites, not only in natural product microbial cultures. Clear indications for performing this study are available [5] but software tailored to predict all the metabolites modifications have not yet been fully developed, established that not only enzymatic transformations but also exogenous compounds from different environmental factors can contribute to production of new metabolites.

We would like to comment some of advantages and challenges of HRMS and its future potential leading to interesting structural information that can direct the an-

alytical work and newly offers perspectives and insights in numerous branches of life sciences.

Mass resolution, mass accuracy and sensitivity make that HRMS instruments and their LC-MS applications cover a wide range of compounds by combining several targeted and untargeted approaches; in addition, fragmentation experiments and/or additional separation power in mass spectrometry (Ion Mobility) provide complementary structure information useful to complete annotations of the molecules. Particularly, accurate mass measurement allows to define elemental formulae, crucial data for an accurate re-identification of known compounds and mostly for the uncover of unknowns (Dereplication).

At any rate, the list of possible elemental compositions often provides numerous results for small molecules too and therefore, the acquisition of further structural data becomes very important.

Whatever the approach to MS/MS (tandem in space or tandem in time), the introduction of multiple-step MS in HRMS is essential to distinguish isomeric compounds and obtain unambiguous results. The basic principle of MS/MS is the selection of precursor ion and its fragmentation, usually by collision-induced dissociation (CID), but different fragmentation strategies are available [6]. It must be taken into account, as it has been known for a long time, that MS/MS fragmentation shows some technical limitations, for instance fragmentation difficulties of compounds and/or their stable adducts, in addition to poor detection of low molecular weight product ions. Another aspect, worthy of being considered, is represented by compounds showing almost identical fragmentation pathways of positional isomers. A few interesting approaches are proposed, as the measure of relative abundances of specific fragmentation ions or the mass analysis of metal complexes [7, 8], but also in this case, since the spectra are strongly affected by the instrumental parameters, the unambiguous identification cannot be obtained. When the compound of interest is present in low abundance and complementary spectroscopic methods, such as NMR, cannot be performed, new ways are required leading to structure elucidation and mass spectrometry provides the solution by the introduction of ion mobility technology. Recent applications using IM-MS (Ion Mobility mass spectrometry) show the emerging contribution of this technique to the discovery of new compounds via the measure of the ion's collision cross section (CCS) parameter which incorporates information about size, shape and charge of a molecule [9-11].

All these recent advances in the analytical field are leading to finding of new antimicro-

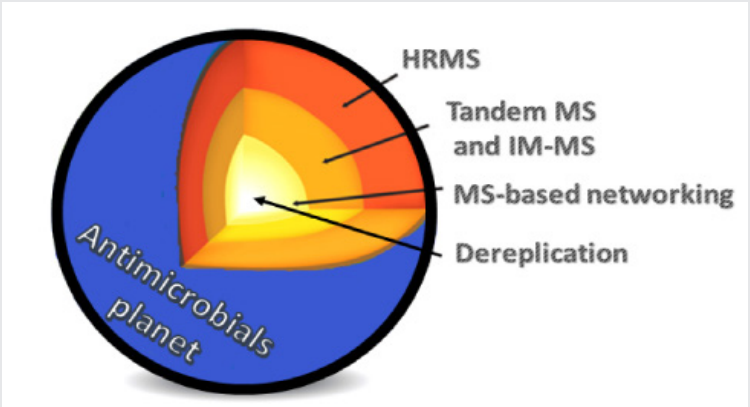

Figure 1. MS tools necessary to get in the core of the antimicrobials identification world.

bial compounds and mostly, to reveal very interesting evidence regarding their structure. Particularly, a plethora of scientific papers suggests and/or demonstrates the role of glycosidic residues in numerous antibiotics.

It had been seen that many biologically active small-molecule natural products produced by microorganisms derive their activities from sugar substituents and changing the structures of these sugars can have a profound impact on the biological properties of the parent compounds [12]. A large number of antibiotics are glycosides and in numerous cases the glycosidic moiety results crucial to their activity, for instance by improving their pharmacokinetic parameters [13]. A series of D-glucosamine derivatives were synthesized and evaluated for their antimicrobial activity, suggesting that the presence of sugar moiety is necessary to biological activity [14]. Sugars or carbohydrates found in human breast milk show properties that can protect against bacterial infections [15].

Our work represented an effective example of novelty determination of a compound exhibiting potential antimicrobial activity via mass spectrometry applications [16]: appropriate combination of HRMS detection and MS/MS fragmentation experiments allowed to obtain the definitive sum formula assignment and identify interesting sugar moieties.

In summary, this letter underlines and stresses the role of advanced HRMS as unique tool in both research and analysis, suggesting that it will play an essential role in discovery and development of carbohydrates based therapeutics, which has been relatively slow so far, mainly due to numerous analytical difficulties. In our opinion, asserted the importance of carbohydrates in several biological events, the recent results can open up new perspectives regarding the preparation and activity correlation of novel glycoside antibiotics.

References

1. Trivella DB, de Felicio R. The Tripod for Bacterial Natural Product Discovery: Genome Mining, Silent Pathway Induction, and Mass Spectrometry- Based Molecular Networking. mSystems. 3:e00160-17 (2018).
2. Kind T, Fiehn O. Strategies for dereplication of natural compounds using high- resolution tandem mass spectrometry, Phytochem Lett 21, 313-319 (2017) .
3. Blaženovic I, Kind T, Ji J, Fiehn O. Software Tools and Approaches for Compound Identification of LC-MS/MS Data in Metabolomics. Metabolites 8(2), 31 (2018).
4. Boiteau RM, Hoyt DW, Nicora CD, Kinmonth-Schultz HA, Ward JK and Bingol K. Structure Elucidation of Unknown Metabolites in Metabolomics by Combined NMR and MS/MS Prediction Metabolites. 8(1), 8 (2018).
5. Lu W, Su X, Klein MS, Lewis IA, Fiehn O, Rabinowitz JD. Metabolite measurement: Pitfalls to avoid and practices to follow. Annu Rev Biochem 86, 277–304 (2017).
6. Nguyen VH, Afonso C, Tabet JC. Comparison of collision-induced dissociation and electron-induced dissociation of singly charged mononucleotides. Int J Mass Spectrom 316-318, 140-146 (2012).
7. Abad-García B, Garmón-Lobat S, Berrueta LA, Gallo B, Vicente F. Practical guidelines for characterization of O-diglycosyl flavonoid isomers by triple quadrupole MS and their applications for identification of some fruit juices flavonoids. J Mass Spectrom 44, 1017–1025 (2009).
8. Pikulski M, Brodbelt JS. Differentiation of flavonoid glycoside isomers by using metal complexation and electrospray ionization mass spectrometry. J Am Soc Mass Spectrom 14, 1437–1453 (2003).
9. Gabelica V, Marklund E. Fundamentals of ion mobility spectrometry. Curr Opin Chem Biol 42, 51-59 (2018).
10. Luzzatto-Knaan T, Melnik AV, Dorrestein PC. Mass spectrometry tools and workflows for revealing microbial chemistry. Analyst 140(15), 4949–4966 (2015).
11. Touilloux R, Joly L, Goscinny S, De Pauw E, Eppe G. Ion mobility-mass spectrometry as a new approach for the screening of pesticide residues in food. Organohalogen Compd 73, 992–994 (2011)

12. Thibodeaux CJ, Melançon CE, Liu HW. Natural-product sugar biosynthesis and enzymatic glycodiversification. Angew Chem Int Ed Engl 47(51), 9814-9859 (2008).
13. Kren V. and Rezanka T. Sweet antibiotics – the role of glycosidic residues in antibiotic and antitumor activity and their randomization. FEMS Microbiol Rev 32(5), 858-889 (2008).
14. Appelt HR, Oliveira JS, Santos RC, Rodrigues OD, Santos MZ, Heck EF, Rosa LC. Synthesis and Antimicrobial Activity of Carbohydrate Based Schiff Bases: Importance of Sugar Moiety. Int J Carbohyd Chem 2013 (2013).
15. Ackerman DL, Doster RS, Weitkamp JH, Aronoff DM, Gaddy JA, Townsend SD. Human Milk Oligosaccharides Exhibit Antimicrobial and Antibiofilm Properties against Group B Streptococcus. ACS Infect Dis 3(8), 595-605 (2017).
16. Carrano L, Naggi A, Urso E. High Resolution Mass Spectrometry for the Recognition and Structural Characterization of a New Antimicrobial Compound. Pharmacol Pharm 9, 135-148 (2018).