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Microbe Metabolic Monitoring – systematic analysis of secondary metabolites by One Strain Many Compounds (OSMAC) methodology and ^{1}H-qNMR of complex mixtures

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To overcome the decreasing number of newly described bioactive compounds in Natural Products Chemistry, new and unexplored natural matrixes have been targeted, aiming to discover biologically promising compounds. Among those, endophytic and rhizospheric microorganisms represent an immensely diverse and unrivaled source of secondary metabolites, since microbe biosynthetic pathways have been optimized by evolution and varies vastly in different substrates.[1] Identifying and quantifying metabolites in a microbe complex matrix is laborious and challenging, since compounds in a biological system range in chemical-physical properties, concentration and structure diversity, creating an overlapped and multivariated data.[2] Moreover, during conventional microbe fermentation, only a fraction of gene clusters is active, leaving behind many other silenced gene that could provide new lead bioactive structures. This work aims to monitor, optimize and quantify bioactive secondary metabolite production in different microbe complex matrixes by OSMAC factorial planning (2^3), followed by compounds quantification and assessment by GSD-IS-^{1}H-qNMR. Specifically, this work provides an accurate measurement of convoluted ^{1}H-NMR signals of important bioactive compound by integrating post-genomic OSMAC strategy[3] and Global Spectral Deconvolution (GSD) algorithm (MestreNova), being the first systematic evaluation of qHNMR quantitation measures in microbe metabolomics. Results show that OSMAC's response to GSD-IS-^{1}H-qNMR quantification is an excellent strategy to monitor metabolite production, enabling optimum metabolic production, doubling target compound concentration. In addition, spectral deconvolution (SD)-based qNMR was reproductive on all species, with results that outperform the comparative methods in both accuracy and precision. For complex matrixes with highly convoluted peaks, GSD-IS-^{1}H-qNMR proved to be unique, allowing the quantification of complex signals with ease and speed not accomplished by classical IS-^{1}H-qNMR and ERETIC-^{1}H-qNMR.

Keywords: qNMR, OSMAC, global spectral deconvolution (GSD).

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