



Alternate non-molecular tools and methods for evaluating physico-chemical and phytohormone responses in biostimulant challenged plants

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Biostimulants including plant growth promoting rhizobacteria (PGPR) are utilized for either crop production or protection and much sought after for their functional benefits in the backdrop of climate change and the pressures of biotic and abiotic stress. The physico-chemical information and structural architecture of biofactors' cell-walls is crucial to understanding complex interactive functions of the biostimulants (algal/plant extracts) or PGPR cell walls and in the overarching extreme environments of soil and plant biomes. Data mining via new generation sequencing tools for gene expression assays served as a good example of molecular tools for deducing the effects of BE on phytohormone responses. Another approach is to employ analytical chemical methods for deciphering biofactor evaluations on phytohormone responses. Thermogravimetric analysis (TGA), Fourier Transform infrared spectroscopy (FTIR), pyrolysis–gas chromatography and mass spectrometry (Py-GC/MS) can be utilized as alternative tools for assessing the physico-chemical characteristics biostimulant formulations (e.g. Rygex, Algavyt, Ryzoset, Manek, Ecoryg and Algavyt Zn/Mn) containing algal/plant extracts, humic and amino acids, lipids and inorganic components, and their storage stability until use on plants. Bioactive compounds identified by FTIR and Py-GC/MS serve as a template for overviewing the fingerprints of known biological activity (e.g. growth hormone, stress mediation, eliciting plant defense or nutrient acquisition etc) of the biofactor formulations. Concomitant Mung bean root and wheat leaf senescence bioassays indicated that among the biostimulants, Rygex, Manek and Ecoryg showed significant auxin like activities while only Manek elicited low level cytokinin activity. Furthermore evaluations of aqueous extracts of processed rye grasses for composites via chemical tools and the plant defence elicitor activity using French bean cell suspension cultures revealed that have the potential to control diseases and stresses in crops. Microbial Biofactors (MBEs) are increasingly becoming common in organic agriculture. However, their mode of actions involved in plant when challenged by environmental stressors is less understood. Current approaches for chemical fingerprinting of bacterial cell wall composition analyses include expensive tools such as cryogenic X-ray photoelectron spectroscopy, spectral snapshots via NMR among others. FTIR and Py –GC-MS have been used for microbial cell wall composites fingerprinting and diagnostic tool in microbiology and food safety. TGA and FTIR tools served as a relatively cheaper and rapid method for evaluating commercial PGPRs (e.g. *Bacillus mucilaginosus*, *B. amyloliquifaciens* and *B. subtilis* Burkholderia sp., *Rahnella aquatalis*) for plant development responses. From the results presented here, we demonstrated that the inclusion of scanning Electron Microscopy facilitated initially to locate the microbial biostimulant at the soil-plant interface and concurrently the combinatorial use of MaxRes TGA–mass spectra profiling of bacterial cells offered further clues to the cell surface chemical composition that is often vital to the efficacy and functional superiority of plant or algal extract biostimulant. Furthermore our proposed TGA tool yields a unique thermal weight loss 'fingerprint' which has the potential for identifying bacterial species and strains at the site of interaction and crucial data sets needed for soil microbiome analyses that complements the molecular data sets that emerge from transcriptome expressions.