



Analysis of phytohormones in vermicompost using a novel combinative sample preparation strategy of ultrasound-assisted extraction and solid-phase extraction coupled with liquid chromatography–tandem mass spectrometry

Hong Zhang^{a,b}, Swee Ngim Tan^c, Chee How Teo^d, Yan Ru Yew^d, Liya Ge^e, Xin Chen^f,
Jean Wan Hong Yong^{a,*}

^a Singapore University of Technology and Design, 8 Somapah Road, Singapore 487372, Singapore

^b Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

^c Natural Sciences and Science Education Academic Group, Nanyang Technological University, 1 Nanyang Walk, Singapore 637616, Singapore

^d School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore

^e Nanyang Environment & Water Research Institute, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, #06-08, Singapore 637141, Singapore

^f College of Life Science, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Article history:

Received 6 December 2014

Received in revised form

11 February 2015

Accepted 15 February 2015

Available online 6 March 2015

Keywords:

Phytohormones

Cytokinins

Auxins

Vermicompost

Ultrasound-assisted extraction

Solid-phase extraction

Liquid chromatography–tandem mass spectrometry

ABSTRACT

Vermicompost (VC), a widely used premium organic fertilizer, is the by-product of symbiotic interactions between earthworms and microorganisms living within them. It has been postulated that phytohormones are plausible “magic compounds” in VC that are responsible for making them such good fertilizers. Thus, a novel approach involving ultrasound-assisted extraction (UAE) and solid-phase extraction (SPE) was developed as a fast and efficient sample preparation method to screen for different classes of phytohormones in VC by liquid chromatography–tandem mass spectrometric (LC–MS/MS) analysis. Nine phytohormones from three different classes, including *trans*-zeatin (*tZ*), kinetin (K), N⁶-[2-isopentyl]adenine (iP), N⁶-benzyladenine (BA), N⁶-isopentenyladenosine (iPR), indole-3-acetic acid (IAA), 4-[3-indolyl]butyric acid (IBA), 1-naphthaleneacetic acid (NAA) and (+)-abscisic acid (ABA), were simultaneously screened. The extraction parameters influencing UAE efficiency were optimized to provide comparable recovery to the conventional mix-stirring (MSt) method. The optimized UAE method was subsequently applied on the analysis of phytohormones in VC, i.e. phytohormone extract was further pre-concentrated and purified using C18 and MCX SPE cartridges prior to LC–MS/MS analysis. The following phytohormones, namely iP, iPR and IAA, were detected and quantified to be 0.49, 0.53, 79.78 ng g⁻¹, respectively; *tZ* was found to be below the limit of quantitation. Recoveries of 10.2%, 9.1%, 18.9% and 0.3% for *tZ*, iP, iPR and IAA were obtained. This is one of the few reported works for the successful detection and quantitation of cytokinins and auxins in VC, that provided the key empirical evidence to explain the growth efficacy of applying VC in promoting plant growth. Additionally, this pioneering work could potentially be applicable for the analysis of other types of organic fertilizers such as composts and activated composted materials awaiting phytohormone analyzes for quality assessment and control.