



Enzymatic valorisation of agro-industrial residues yields DAMP-enriched biostimulants for sustainable crop protection

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The agri-food industry generates every year a considerable amount of waste residues (WR) that negatively impacts the environment and the economic well-being of people. For this reason, there is an urgent need to convert these by-products into value-added compounds. In parallel, efforts to reduce the use of synthetic pesticides have accelerated the development of natural biostimulants that enhance crop performance in an environmentally sustainable manner^{1,2}. Among emerging technologies, enzyme-assisted extraction represents a greener alternative to traditional non-enzymatic extraction, with benefits in terms of reduced solvent consumption and improved process scalability³. In this study, different agro-industrial WR were subjected to enzymatic hydrolysis to obtain oligosaccharide-enriched mixtures that were tested for their bioactivity. Thermal alkaline pretreatment was applied to remove lignin, followed by enzymatic hydrolysis of the pretreated WR using commercially available endo-glycosyl hydrolases (e.g., endo-1,4- β -D-glucanase and endo-1,4- β -xylanase) and in-house produced xylanases. The obtained hydrolysates were analysed via HPAEC-PAD, showing the presence of both low molecular weight oligosaccharides of xylose (degree of polymerization 2-6) and unidentified peaks, likely related to higher molecular weight oligosaccharides. These mixtures contain cell wall-derived oligosaccharides that act as damage-associated molecular patterns (DAMPs), capable of eliciting plant immune responses⁴. Oligosaccharide profiling revealed that xylanase-treated rice husk produced the most enriched hydrolysate, characterized by elicitor-active xylo-oligosaccharides. The different hydrolysates were applied to *Arabidopsis thaliana* to assess their ability to induce defence priming. Consistently, the xylanase-treated rice husk hydrolysate showed the highest efficacy, significantly enhancing resistance against the necrotrophic pathogens *Botrytis cinerea* and *Pectobacterium carotovorum*. Gene expression analyses confirmed strong activation of defence-related markers in treated plants, with no direct antimicrobial effect on the pathogens. Overall, these findings highlight the potential of DAMP-enriched biostimulants derived from enzymatically treated lignocellulosic waste as a sustainable strategy for crop protection.

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