Immunomodulatory Fungal Polysaccharides: Prospective Candidates for the Development of New Nutraceuticals and Adjuvants

Namal Perera[a,b,c], Fang-Ling Yang[c], Kuo-Feng Hua[d] and Shih-Hsiung Wu[c,e]

[a] *Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Belihuloya, Sri Lanka*
[b] *Department of Chemistry, National Tsing-Hua University, Hsinchu, Taiwan*
[c] *Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan*
[d] *Department of Biotechnology and Animal Science, National Ilan University, Ilan, Taiwan*
[e] *Department of Chemistry and Institute of Biochemical Sciences, National Taiwan University, Taiwan*

Naturally occurring complex carbohydrates represent a structurally diverse group of macromolecules which shows a broad range of biological activities. However, their clinical contribution is still limited because of the structural complexity, lack of studies on structure activity relationship and underlying mechanisms of biological activity of individual polysaccharide components. In the present study polysaccharides were isolated from the cold-water extracts of two mushroom species, Antrodia cinnamomea and Auricularia auricula-juadae, to investigate their immunostimulatory potential and mechanisms of action. Polysaccharides were purified by size exclusion and ion exchange chromatography and their chemical structures were elucidated by Gas Chromatography-Mass Spectroscopy (GC-MS) and Nuclear Magnetic Resonance Spectroscopy (NMR). Immunostimulatory properties of each polysaccharide were investigated targeting the proinflammatory cytokines (TNF-α, IL-6) and nitric oxide (NO) producing abilities in selected immune cell models (J774.1A and Raw 264.7). Immunologically active polysaccharide of *A. cinnamomea* (**ACP**; MW>70 kDa) was chemically identified as galactomannan, with branched octasaccharide repeating units, whereas polysaccharide of *A. auricula-ju dae* (**AAPS**, MW>70 kDa) was identified as glucuronoxylomannan with a heptasaccharide repeating units. O-acetyl modification at mannose and xylose residues was observed in **AAPS** while glucuronic acid residues remained unacetylated. Both **ACP** and native **AAPS** activated murine macrophage cells to produce TNF-α and IL-6 in a concentration dependent manner. Toll like receptor 4 was identified as the main receptor involved in **ACP** and **AAPS** mediated macrophage activation. Complete abrogation of immunostimulatory properties was observed in deacetylated and carboxyl reduced **AAPS** indicating the essentiality of both acetyl and carboxylic functionalities in immunostimulatory process. Molecular modelling data further demonstrated the role of acetyl and caroboxyl moieties in receptor binding. Our findings have provided firm scientific evidences for the immunoenhancing properties of two mushroom species and the potential of these two polysaccharides to be strong candidates for the development of new carbohydrate-based nutraceutical supplements and adjuvants in the treatment of immunity related disorders in near future.

Keywords: *Antrodia cinnamomea*, *Auricularia auricula-juadae*, immunostimulatory properties, Polysaccharides