**SUMMARY**

In this study, polysaccharides from *Xanthoria parietina*, a wide spread lichen from central Europe, were isolated, purified and analyzed concerning the structural features, in combination with functional investigation of the influence on murine macrophage cell line RAW264.7 *in vitro*.

Defatted dried lichen (140 g, $W_T$) was submitted to consecutive cold water (4°C, 12 h), freezing (-20°C) and thawing (room temperature), hot water (100°C) as well as alkaline (0.5 M aq. NaOH) extractions (each in triplicate). Only hot aqueous extract, XH (2.8%, w/$W_T$) was further fractionated by ion exchange chromatography to yield mainly H-1 (0.7%) and H-2 (0.4%) (w/$W_T$) fractions. Chromatographic purification of the fractions by gel permeation chromatography yielded polysaccharides H-1-3 (0.39%) and H-2-1 (0.07%) (w/$W_T$) with molecular weights $M_w$ of 13.7 and 525 kDa respectively, prior to ethanol precipitation, dialysis and lyophilisation.

Monomers composition analysis by HPAEC-PAD and GC-MS revealed that H-1-3 consisted mainly of glucose (> 98%), while H-2-1 was composed of mannose (74.3%), galactose (14.1%), glucose (9.8%) and rhamnose (1.8%) as well as trace of arabinose. D-/L-configuration of diastereomers analyzed by capillary zone elactrophoresis (CZE) as well as the anomeric configuration by $^1$H- and $^{13}$C-NMR indicated the presence α-D- glucose in H-1-3 and α-D-mannose, α-D-galactose, α-D-glucose and α-L-Rhap in H-2-1. Subsequently, linkage sequences were determined by GC-MS analysis of partially methylated alditol acetates, nano-ESI QTOF-MS/MS analysis of oligosaccharides and 2D-NMR analysis of native polysaccharides and oligosaccharides. In nano-ESI-Q-TOF MS/MS analysis, the use of silver adducts in positive-ion mode settings was established and found to be capable of rising A- and X-type fragment ions generated from cross-ring cleavages, together with the rise of B- and Y-type ions from the glycosidic bonds cleavages of the oligosaccharides prepared from H-1-3 but not H-2-1. For H-2-1, the use of chloride adduct in negative-ion mode settings was better capable in rising similar type of fragment ions.

As a result, the structure of H-1-3 was elucidated to be an α-glucan with α-D-Glc*p*-(1→[→[4)-α-D-Glc*p*-(1]$_2$→[6)-α-D-Glc*p*-(1]$_3$→4)]$_n$ core backbone. The (1,4)- and (1,6)-α-D-Glc*p* linkages were in a 2:3 molar ratio. Thereafter, H-2-1 was characterized to be a galactofuranomannan with →[6)-α-D-Man*p*-(1→[2,6)-α-D-Man*p*-(1]$_2$→[2)-α-D-Man*p*-(1]$_2$→]$_n$ core units and main side chains of β-D-(1,3)-Gal*f* linked at *O*-6 to →2)-α-D-Man*p*-(1→, together with minor terminal units of α-D-(1,4)-(1,6)-Glc*p* units attached to core chain at *O*-6 position and α-L-Rha*p* linked to Gal*f* side chain at *O*-2 position (Man*p*: Gal*f*: Glc*p*: Rha*p* linkages ratios = 9:3:2:1).

For *in vitro* functional investigation, LPS-depleted H-1-3 and H-2-1 were used. Within a cellular uptake study, FITC-H-1-3 and FITC-H-2-1 were found to be rapidly internalized (15–30 min) into RAW264.7 macrophages. Compared to H-1-3, clear attachment of H-2-1 on the cell surface was observed. H-1-3 and H-2-1 showed no toxic effect and exerted significant stimulatory effects by enhancing the phagocytosis of FITC-Zymosan by up to 31% ($p < 0.05$). The polysaccharides displayed weak influence on RAW264.7 NO production but in contrast significantly enhanced interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) secretion in a dose dependent manner ($p < 0.05$). In lipopolysaccharide (LPS)-stimulated RAW264.7 (0.5 μg/mL), both compounds suppressed NO production and iNOS secretion. In addition, H-1-3 and H-2-1 also inhibited LPS-stimulated IL-1β induction by up to 50% where only H-1-3 showed inhibition in dose-dependent manner at concentrations range of 1.0 to 100 μg/mL ($p < 0.05$). However, no influence was observed on LPS-stimulated TNF-α. This study also revealed mannose receptor, Dectin-2 expression by RAW254.7 macrophages and the potential interaction of H-2-1 with Dectin-2. Dectin-2 antibody also reduced the binding affinity of H-2-1 to cell surface.

Summarizing, this study purified and identified two new polysaccharides from *X. parietina*, one uncommon α-glucan, H-1-3 as well as a new galactofuranomannan, H-2-1. Both polysaccharides displayed various influence on cell physiology of murine macrophages *in vitro* which until now have not yet been reported.