Inonotus obliquus (Chaga) – a white rot fungus found on birch trees in the northern hemisphere – has been used in traditional medicine in Europe and Asia for centuries. Native peoples have made use of Chaga by brewing it as a tea to treat gastrointestinal problems, to heal wounds and even to treat cancer. The last few decades, studies have found Chaga to contain biologically active substances such as polysaccharides, triterpenoids, polyphenols and melanin. In vivo effects such as tumor growth inhibition have been observed in mice receiving various Chaga extracts. The main hypothesis behind the tumor inhibiting effect is two-fold: i) fungal polysaccharides may inhibit tumor growth indirectly by activating certain immune cells such as macrophages and ii) triterpenoids and other steroids from Chaga may give a direct cytotoxic effect against cancer cells. While triterpenoids from Chaga have been extensively characterized, detailed analysis of the polysaccharides is lacking. The present work has aimed to isolate and characterize the polysaccharides in Chaga, by e.g. column chromatography (ion exchange/gel filtration), GC-MS, SEC-MALLS and extensive NMR analysis. The water-soluble polysaccharides were found to be complex hetero-polysaccharides, with a structure dominated by (1→3)/(1→6)-β-glucan and (1→6)-α-galactan, with β-xylose, α-mannose and α-galacturonic acid present in significant amounts. 3-O-methyl α-galactose was reported in Chaga for the first time. The polysaccharide fractions obtained were screened in in vitro bioassays for their potential as immunomodulators. Several fractions showed promising results by activating murine bone marrow derived macrophages to inhibit the growth of Lewis lung carcinoma cells in vitro. Theresults suggest further studies to be conducted on immune cell activation and in vivo tumor growth inhibition.