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Current advancement on submerged cultures of *Inonotus obliquus* ----- problems, strategies and prospectives

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Abstract: The medicinal fungus *Inonotus obliquus* (Fr.) Pilat (*Hymenochaetaceae*) has been used as a folk remedy in Russia and Eastern Europe for more than four centuries (Zheng et al. 2007b), where its beneficial influence on the treatment of several human diseases (Chen et al. 2007) in the absence of unacceptable toxic side effects, has become established (Huang 2002; Saar 1991). *I. obliquus* usually occurs as a sterile conk (sclerotium) called a 'Chaga' on the trunks of *Betula* trees (Campbell and Davidson 1938). It produces a diverse range of metabolites including polysaccharides, triterpenoids and steroids. Among these are biologically active compounds possessing hypoglycaemic (Mizuno et al. 1999), hepato-protective (Slomon and Alexander 1999), anti-fungal (Kahlos 1994) and antitumor activities (Kahlos et al. 1987). Their ability to inhibit viral replication has also been reported (Ichimura et al. 1998). Furthermore, Chaga synthesizes a range of phenolic constituents, which include small phenolics (Nakajima et al. 2007), hydrolysable tannins (Yang and Zheng 2007), flavonoids (Zheng et al. 2007a), polyphenols (Lee and Yun 2007) and melanins (Babitskaia et al. 2000). These phenolic compounds show a remarkable potential for scavenging free radicals (Babitskaia et al. 2000; Cui et al. 2005; Nakajima et al. 2007), and thus reduce the incidence of oxidative stress-induced diseases (Nakajima et al. 2007), including cancer (Orzechowski 2007), hypertension (Kwon et al. 2007) and neurodegenerative (Alzheimer's and Parkinson's diseases) (Heo and Lee 2005) and autoimmune diseases (Galli et al. 2005). Consequently, potentially they have considerable pharmaceutical importance. In nature, however, this fungus is restricted to cold habitats (45° N -50° N latitude) and grows very slowly, suggesting that the naturally occurring sclerotial form is not a reliable source of these compounds (Zheng et al. 2007a). In the past decades submerged culture of *Inonotus obliquus* experienced three stages. In first stage the culture of *I. obliquus* was targeted for the accumulation of mycelial biomass or polysaccharides with no consideration in accumulating secondary metabolites; while in the second the culture of the fungus was targeted for accumulating melanins, or total polyphenols, lanostane-type triterpenoids with the emphasis

on culture conditions favorable to their accumulation. These studies detailed partially the features of secondary metabolisms in submerged cultures of the fungus. However, the bioreactors used for the culture were shake flasks, where culture parameters can neither be monitored nor controlled. In addition, previous evaluation on submerged cultures was based either on total phenolic compounds, or total triterpenoids or melanins. It is not clear how the carbon and nitrogen sources are partitioned particularly to the biosynthesis of secondary metabolites. More importantly, the products from submerged cultures have not yet been undergone a systemic pharmacological assays. Submerged cultures of *I. obliquus* in third stage were conducted in constantly stirring tank reactor, where parameters in fungal process were able to be recorded. In these studies, integrated analytical methods were utilized to measure the production of melanins, total phenolic compounds and their compositions and physiochemical responses to H_2O_2-induced oxidative stress in terms of accumulation of phenolic compounds and expression of antioxidant enzymes. Using NMR-based Metabolomic analytical methodology, recently published works by Zheng et al (2009 a & b) even uncovered the light conditions favorable to the biosynthesis of hispidin analogs and mechanisms of fungal elicitor-enhanced accumulation of polyphenols with chemical profiles similar to those in sclerotia. These findings have gained some insights into regulation on accumulation of bioactive metabolites in submerged cultures of *I. obliquus*.

Key words: *Inonotus obliquus*, submerged cultures, regulation on metabolites, pharmacological activities

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