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学位論文題目　Effects of Fluorides and Nanobubble Water on the Immunomodulatory and Antioxidative Activities of Polysaccharides from *Cordyceps militaris*
（サナギダケ多糖類の免疫調節および抗酸化活性に対するフッ素化合物とナノバブル水の効果）

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論　文　の　要　旨
Abstract of thesis

Cordyceps militaris has traditionally been used as medicine for hundreds of years in some Asia countries. Previous research has shown its significant pharmacological properties on a variety of diseases and functional food application. The polysaccharides of *C. militaris* possess various activities, such as immunomodulatory, antioxidant and anti-tumor activities. Many countries especially some Asia countries have serious fluorine pollution problems. Urban fluorine pollution mainly damaged surface plants, fungi and other organisms through groundwater. Fluorine accumulated in surface water, and it will cause many diseases such as dental and Skeletal fluorosis when the intake of fluorine exceeds a safe dose. Nanobubble water (NBW) can promote internal pressure, gas stability, high gas dissolution rate results in mass transfer, chemical reactions. Up to now, however, little information is available regarding the effect of NBW-based fermentation on mycelia of *C. militaris*.

In this study, the liquid fermentation system was first established. The strain was submerged in culture medium for fermentation, to which fluoride and NBW were supplemented at different concentrations. The crude polysaccharides were extracted with boiled water for 3 hours, and then precipitated 3 times. Extracts from the optimal conditions were selected for further purification with their antioxidant and immunomodulatory activities being evaluated. The main results can be concluded as follows.

This dissertation is divided into 4 chapters. In Chapter 1, the author first introduced the background of the research, including polysaccharides, fluoride, nanobubbles, the chemical structure, antioxidant and immunomodulatory activities of polysaccharides. Then the author arrived at the research target and thesis structure. In Chapter 2, the author established the new fermentation system induced by fluoride,

and the crude polysaccharides from *C. militaris* were purified with their bioactive effects being evaluated. Results show that supplementation of KF 1.0 mg/L during the *C. militaris* fermentation was beneficial for the mycelia accumulation, reaching the highest concentration of 3.4 mg/mL. And the crude polysaccharide extracts exhibited a higher DPPH scavenging activities and macrophage proliferation activity (123.04%). After purification, three major fractions were obtained from the crude polysaccharides in B2 group, with the strongest SOD activity (27%) and macrophage proliferation activity (122.01%) in CMPF-3 all the extracts. In Chapter 3, the author investigated the effects of NBW supplementation on biomass accumulation of *C. militaris* and the antioxidant and immunomodulatory activities of extracted polysaccharides. The highest mycelium concentration (3.90 mg/mL) and crude polysaccharides extraction yield (12.76%) were obtained in 25%-NBW group. The antioxidant activities of mycelia were significantly promoted after supplementation with NBW. The polysaccharides from 25%-NBW, 75%-NBW, and 50%-NBW groups exhibited the strongest scavenging activities on DPPH and ABTS radicals, and strongest reducing power, respectively, achieving the highest radical scavenging rate (nearly 100%) at 1.2 mg/mL, the lowest IC_{50} value (1.09 mg/mL) and the highest OD value (2.13) at 2.0 mg/mL, respectively. The author also noticed that G2/M phase was significantly increased in the 25% NBW group, with the maximum percentage of 9.67%. 25%-NBW extracts exhibited an obvious proliferation effect on macrophage cells, with the highest viability (123.6±6.9%) being achieved at 6.25 μg/mL. When compared with the untreated cells, the highest IL-2, IL-4, and IL-10 production in the 25%-NBW treated groups were 180.4, 86.7, and 152.2 pg/mL, respectively. In addition, the fluorescence intensity in cells treated with 25%-NBW was significantly increased in a dose-dependent manner in comparison to the control group, demonstrating that 25%-NBW treatment may mediate the upregulation of intracellular reactive oxygen species (ROS) production. Finally, in Chapter 4, the author summarized the major conclusions, and proposed the future research directions.

審　査　の　要　旨

Abstract of assessment result

Natural *C. militaris* is rare and expensive, thus it's very important to obtain the bioactive compounds by artificial culture. This study examined the effects of fluorides and NBW on the polysaccharides extracted from the mycelia of *C. militaris* after liquid fermentation. Results show that supplementation with 0.1 mg/L NH_4F and 1.0 mg/L KF benefited the *C. militaris* biomass accumulation. The highest crude polysaccharide extract was yielded at 10.0 mg/L KF, which demonstrated more effectiveness on RAW264.7 cell proliferation, and all the extracts exhibited strong antioxidant and immunomodulatory activities on CMPF-3. In the NBW-based fermentation, the highest mycelium concentration (3.90 mg/mL) and crude polysaccharides extraction yield (12.76%) were obtained in the 25%-NBW group, which exhibited the strongest DPPH radical scavenging activity, and significantly increased the G2/M phase as well, reaching the maximum percentage of 9.67%. The 25%-NBW treated groups also produced obviously higher IL-2, IL-4, and IL-10 than the untreated groups, which was the same as ROS production assay. More discussion on the results and analysis of the mechanisms are necessary to shed light on the observed phenomenon, and combination use of fluoride and NBW in *C. militaris* fermentation may be one more promising alternative.

The final examination committee conducted a meeting as a final examination on 15 January, 2021. The applicant provided an overview of the dissertation, addressed questions and comments raised during Q & A session. All the committee members reached a final decision that the applicant has passed the final examination.

Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Environmental Studies.