学位論文内容の要旨

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学 位 論 文 題 目

Studies on the Potential of Marine Brown Algae
for Antimicrobials and Mineral Supplements
（海産褐藻の抗菌およびミネラルサプリメント機能に関する研究）

Since marine algae have great biodiversity, it has been explored as novel resources to use functional ingredients for human health. In current researches especially in brown algae, polyphenols, sulfated polysaccharides and carotenoids from the brown algae have been shown to possess multiple physiological activities, including anti-allergic, anti-coagulant, antihypertensive, anti-inflammatory antimicrobial, anti-oxidative, and anti-tumor and anti-viral activity. With the potential of marine algae as natural resources, this study was conducted to investigate the potential of marine brown algae as natural candidates for antimicrobials or mineral supplements.

In Chapter 1, antimicrobial activity of an unexplored marine alga *Cystoseira hakodatensis* was screened against some food pathogenic and spoilage bacteria, and major antimicrobial compound was isolated and characterized. Within crude extracts prepared with different solvents, a crude methanol extract showed inhibitory effects on the growths of *Bacillus cereus* and *B. licheniformis*. To isolate the major antimicrobial compound, a sequential active-guided isolation was applied: liquid-liquid extraction, column chromatography, bio-autography and RP-HPLC. However, the antimicrobial activity decreased during the isolation procedure using HPLC. A marked antimicrobial compound (active α) was isolated in hydrophobic fraction (F1, hexane-ethyl acetate, 75:25, v/v) on the bio-autography, and it was determined as phenolic compound without carbohydrates and proteins. Regarding the antimicrobial potential, the active α showed stronger antimicrobial abilities against *B. cereus* and *B. licheniformis* in comparison to epigallocatechin gallate. These results indicated that *C. hakodatensis* is a potential source of antimicrobial agents to prevent the growth of these two bacteria.

In Chapter 2, antimicrobial activity of polysaccharides from marine brown algae, including alginate, laminarin and fucoidans, was determined, and especially anti-biofilm of fucoidan from marine alga *Fucus vesiculosus* and its mode of actions were investigated. Among the tested polysaccharides, the fucoidan from *Fucus vesiculosus* (fucoidan F) showed the best inhibitory effect against all tested Gram-positive bacteria, including *Bacillus cereus, B. licheniformis, B. subtilis, Listeria monocytogenes and Staphylococcus aureus*, and also, on their biofilm formation, *B. subtilis* was mostly affected by the fucoidan F, the biofilm formation completely suppressed. In mode of the action against *B. subtilis*, cell lysis of the bacteria wasn't occurred by treating with fucoidan F at concentration of 0.63-2.5 mg/ml for

12 h. To evaluate the effect of fucoidan F on the cellular surface of *B. subtilis*, the adhesion of fucoidan F to the bacterial cells, and the cellular surface charge and hydrophobicity were determined. In the adhesion, the amount of fucoidan F attached to the cells showed a concentration-dependent manner, it was increased by the added concentration. The binding of cytochrome C on the cells was elevated by fucoidan F, indicating the cells negatively charged. In contrast, the cellular surface hydrophobicity was notably decreased by fucoidan F. On the morphological observation by SEM, the adhesion of fucoidan F to the bacterial cells was demonstrated. However, cells destruction wasn't induced.

In Chapter 3, the potential of the marine alga *Sargassum horneri* for calcium supplements was evaluated. To prepare the extract containing high calcium content from the algae, different solvent extracts were prepared, and the optimal condition was investigated using a central composite design (CCD) of response surface method (RSM). Through the CCD, totally 18 experimental trials were built, and the calcium recovery was mostly affected by the lactic acid concentration. The optimal condition was found to 0.1% lactic acid, 86.95 °C and 8.99 h. The effect of lactic acid extract (LAEx) on bone formation in 48 female Sprage-Dawley rats was investigated for 4 weeks of their growth phase. The rats were divided into four groups based on diet: two calcium-sufficient and two calcium-deficient diets. A normal control (N) was fed with AIN-93G diet; the normal diet plus 1 % LAEx (N+E); a calcium-deficient control (CD); the calcium-deficient diet plus 1 % LAEx (CD+E). Bone formation in the rats was evaluated using the wet weight, length, diameter, and bone mineral density (BMD) of the femur. Serum parameters were also examined. The feed intake among the four groups didn't differ significantly ($p<0.05$). N+E gained the most body weight, while CD gained much less weight than the other groups. Regarding the calcium absorption, there was no significant difference in calcium-deficient groups (CD and CD+E), but in normal diet groups, N+E was significantly higher than N ($p<0.05$). The BMDs in the normal groups were not significantly different ($p<0.05$), while in the calcium-deficient groups the BMD was significantly higher in CD+E than in CD ($p<0.05$). The serum calcium and phosphorus levels in all groups were not associated with markers of bone growth related to the addition of LAEx. The osteocalcin content and alkaline phosphate (ALPase) activity were higher in the calcium-deficient groups than in the normal groups ($p<0.05$). Ultimately, the osteocalcin content and ALPase activity were lower in CD+E compare to CD.

Although the bioactivity of algal components from selected marine brown algae was partially confirmed in this study, but in terms of antimicrobial activity, the phenolic compound isolated from *C. hakodatensis* and fucoidan from *F. vesiculosus* had the potential as antimicrobials or bio-preservatives against *Bacillus* species. In addition, the calcium absorption promoting property *in vivo* of the lactic acid extract from *S. honeri* suggests possibility as candidate for mineral supplements. This study was conducted to investigate the potential of algal components from marine brown algae as candidate for antimicrobial agents or mineral supplements.