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学位論文審査の要旨

博士の専攻分野の名称　博士（農学）　氏　名 Mohammad Nazrul Islam Bhuiyan

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| 審査担当者 | 主査 | 特任教授 | 生方　信 |
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Growth factors for uncultured bacteria and structural requirements of quercetin for inhibiting advanced glycation end products (AGEs) formation
(難培養バクテリアに対する増殖因子並びに
糖化反応阻害物質としてのケルセチンの構造要求性)

The thesis consists of two main topics including 66 figures, 6 tables and one appendix. The growth of strain ASN212 related to *Leucobacter* sp. was stimulated by the supernatant of *Sphingopyxis* sp. strain GF9. In this study, novel porphyrin type growth factors produced by strain GF9 were identified to induce vigorous proliferation of a previously uncultured bacterial strain ASN212 at pico- to nanomolar levels. Even more surprisingly, the growth factors showed self-toxicities against the growth factor producing bacterium, strain GF9 at the concentrations of pico- to nanomolar levels. The second part of this study is the elucidation of structure-activity relationships for the dietary flavonoid, quercetin and its structural requirements for inhibition of advanced glycation end products (AGEs) formation. The study revealed that quercetin can rapidly trap methylglyoxal (MGO) by forming mono- and di-MGO adducts under the oxygen-free conditions. The positions C6 and C8 on the A ring of quercetin were the major active sites for trapping MGO, which comprise the essential structural requirement for quercetin to inhibit AGEs formation.

**1. Identification of strain ASN212 growth factors produced by strain GF9**

Production of growth factors for strain ASN212 was achieved by a 72-h culture of strain GF9 in a 100 L stirred tank bioreactor (three batches). The active MeOH soluble fraction (dried, 150 g) obtained from 210 litters of culture broth was subjected to Diaion HP20 column chromatography to give a MeOH eluting fraction, which induced growth of strain ASN212 at equivalent concentration (EC). Further bioassay guided fractionations by Sephadex LH-20 column chromatography led to the identification of the active fractions. Subsequent preparative ODS HPLC gave six different growth factors **A-F**, which were identified as zinc coproporphyrin I (0.8 mg), coproporphyrin I (0.3 mg), zinc coproporphyrin III (zincphyrin, 3.0 mg), coproporphyrin III (1.2 mg), zinc coproporphyrin I monomethyl ester (zincmethylphyrin I, 0.8 mg), zinc coproporphyrin III monomethyl ester (zincmethylphyrin III, 2.0 mg). Minimun effective concentrations (MECs) were

determined by measuring the dry weight of bacterial cells from a 70-ml culture of strain ASN212 stimulated by each individual growth factor. The growth inducing activity of growth factor **C** was most evident with a MEC value of 10 pg/ml (14 pM) followed by growth factor **D (**1 ng/ml), **F** (3.5 ng/ml), **E (**7 ng/ml), **A (**14 ng/ml), **B (**25 ng/ml), respectively. A panel of commercial and synthetic porphyrins was tested to explore the generality of porphyrin as growth factor for strain ASN212, while coproporphyrin I dihydrochloride, coproporphyrin III dihydrochloride, coproporphyrin III tetramethyl ester, hemin and hematin showed significant growth stimulation of strain ASN212. Two growth factors **E** and **F** named as zincmethylphyrin I and zincmethylphyrin III were found to be new porphyrin derivatives. It was also noteworthy that all six growth factors completely inhibited the growth of strain GF9 itself. This research has broken new ground by demonstrating that coproporphyrins produced by strain GF9 or commercially available porphyrins could have an impact on the growth of previously uncultured bacterial strain in laboratory conditions. This study suggests that coproporphyrins function as a novel type of signal molecules across a boundary at phylum level in the complex bacterial communities.

**2. Structural requirements of quercetin for inhibiting AGEs formation**

Structure-activity relationship studies on fourteen commercial flavonoids indicated that quercetin was the most potent inhibitor against AGEs formation in glucose-, ribose- or MGO-mediated bovine serum albumin (BSA) assay systems. To address the structural requirement of quercetin as a potent inhibitor of AGEs formation, BSA was incubated with MGO under both in conventional and oxygen-free conditions. The reaction of quercetin with MGO in the presence of BSA yielded four different products. The structures of these compounds were determined to be quercetin-MGO diadduct, two different quercetin-MGO monoadducts, and 3,4-dihydroxybenzoic acid. 3,4-Dihydroxybenzoic acid should be formed by an oxidative degradation of A and C rings of quercetin, and no other degradation products were detected. Under the conventional conditions, MGO accelerated the oxidative degradation of quercetin to form 3,4-dihydroxybenzoic acid. However, the diadduct formation of quercetin with MGO was accelerated under oxygen-free conditions, whereas the degradation of quercetin was suppressed. The C6 and C8 positions of the A ring in quercetin were the major active sites for trapping MGO to form both mono- and diadducts. These results might provide a new insight to prevent diabetes complications and aging caused by AGEs formation.

The first and most important topic of the thesis is the identification of coproporphyrins including structurally new zincmethylphyrins as growth factors for previously uncultured bacterium. These findings would have an impact on microbial chemistry and related fields. Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.