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学 位 論 文 要 旨
(Summary of the Doctoral Dissertation)

学位論文題目 (Dissertation Title)	Study on Amino Acid and Lipid Metabolism Accountable for Food Flavor (食品香気に寄与するアミノ酸代謝および脂質代謝に関する研究)
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Not only taste but also flavor has a significant impact on the "deliciousness" of food as perceived by people. Flavor characteristics of food have a significant impact on its palatability and influence the qualitative value of the food itself. In this study, the molecular mechanisms of biosynthesis of flavor compounds derived from amino acids in soybean (*Glycine max*) and fatty acids in mushrooms were elucidated.

In Chapter 1, I identified the characteristic aromatic properties of soybean due to sulfur-containing amino acids. Several soybean germplasms, such as Nishiyamahitashi 98-5 (NH) among local varieties in Nagano Prefecture, have an intense seaweed-like flavor after cooking because of their high seed *S*-methylmethionine (SMM) content. In this study, I compared the amounts of amino acids in the phloem sap, leaves, pods, and seeds between NH and the common soybean cultivar Fukuyutaka. This revealed a comparably higher SMM content alongside a higher free L-methionine (L-Met) content in NH seeds, suggesting that the SMM-hyperaccumulation phenotype of NH soybean was related to L-Met metabolism in seeds. To investigate the molecular mechanism behind SMM hyperaccumulation, I examined the phenotype-associated gene locus in NH plants. Analyses of the quantitative trait loci in segregated offspring of the cross between NH and the common soybean cultivar Williams 82 indicated that one locus on chromosome 10 explains 71.4% of SMM hyperaccumulation. Subsequent fine-mapping revealed that a transposon of about 6 kbp insertion into the intron of a gene, *Glyma.10g172700*, is associated with the SMM-hyperaccumulation phenotype. The *Glyma.10g172700*-encoded recombinant protein showed Met-γ-lyase (MGL) activity in vitro, and the transposon-insertion mutation in NH efficiently suppressed *Glyma.10g172700* expression in developing seeds. Exogenous administration of L-Met to sections of developing soybean seeds resulted in transient increases in L-Met levels, followed by continuous increases in SMM concentrations, which was likely caused by L-Met methyltransferase activity in the seeds. Accordingly, we propose that the SMM-hyperaccumulation phenotype is caused by suppressed MGL expression in developing soybean seeds, resulting in transient accumulation of L-Met, which is converted into SMM to avoid the harmful effects caused by excess free L-Met.

In Chapter 2, I studied the biosynthesis mechanism of 1-octen-3-ol, the main volatile component of mushrooms. 1-Octen-3-ol is a volatile oxylipin found ubiquitously in Basidiomycota and Ascomycota. As 1-octen-3-ol attracts mosquitoes and flies, its involvement in emitter–receiver ecological communication has been proposed. Although the biosynthetic pathway to form 1-octen-3-ol from linoleic acid through linoleic acid 10(*S*)-hydroperoxide has been proposed in mushrooms, the

enzymes involved in this pathway have not been identified. I determined that the *Coprinopsis cinerea* dioxygenase 1 and 2 (CcDOX1 and CcDOX2) genes in the mushroom *C. cinerea* contains an N-terminal cyclooxygenase-like heme peroxidase domain and a C-terminal cytochrome P450-related domain. Through analysis of products formed from linoleic acid by the recombinant CcDOX1 and CcDOX2 proteins, I found that CcDOX1 preferentially catalyzes to form the 10(*S*)-hydroperoxide of linoleic acid (10*S*-HPODE), meanwhile CcDOX2 form the 8-hydroperoxide of linoleic acid. Moreover, disruption of *Ccdox1* in *C. cinerea* (Δ*Ccdox1*) mycelia suppressed 1-octen-3-ol synthesis. Administration of the 10*S*-HPODE to the microsome fraction prepared from mycelia resulted in the efficient production of 1-octen-3-ol. Together, these results indicate that CcDOX1 is essential for the biosynthesis of 1-octen-3-ol as the oxygenase that forms 10*S*-HPODE, followed by the cleavage enzyme.

I studied physiological and ecological significance of 1-octen-3-ol of mushroom. Δ*Ccdox1* was less attractive to fruit fly larvae, while the feeding behavior of fungus gnats on Δ*Ccdox1* mycelia showed little difference from that on the mycelia of the wild-type strain. The proliferation of fungivorous nematodes on Δ*Ccdox1* mycelia was similar to or slightly worse than that on wild-type mycelia. Thus, 1-octen-3-ol seems to be an attractive compound for some animals that interact with mushrooms.

(様式9号)

学位論文審査の結果及び最終試験の結果報告書

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【論文審査の結果及び最終試験の結果】

本論文では食品フレーバーに寄与する香気成分生成代謝経路に関する研究成果を示している。第1章では長野県在来ダイズ品種のユニークな海苔フレーバーの前駆体、*S*-メチルメチオニン生合成代謝経路に関して検討し、当該ダイズ品種と普通ダイズの掛け合わせ後代でのポジショナルクローニングによりメチオニンγ-リアーゼ遺伝子のイントロン部分に *Copia* 型トランスポゾンが挿入されることで遺伝子発現が抑制されているためにメチオニン代謝が撹乱されていることが原因であることを明らかにした。更に当該遺伝子の発現様式を検討し、登熟中のダイズ種子での遺伝子発現抑制が *S*-メチルメチオニン蓄積に寄与していることを明らかにし、登熟中ダイズ種子への ^{13}C 標識メチオニンのフィード実験によりその妥当性を確認した。第2章ではキノコの特徴的なフレーバー成分である1-オクテン-3-オール生合成機構解明に挑戦し、モデル担子菌ウシグソヒトヨタケのゲノム情報からリノール酸酸化酵素 CcDOX1 遺伝子を発見、その遺伝子破壊株を調製して *CcDOX1* が1-オクテン-3-オール生合成に必須であることを明らかにした。更に、CcDOX1 を昆虫細胞で発現させ、リノール酸からの生成物を LC-MS/MS 分析し、その生成物がリノール酸 10*S*-ヒドロペルオキシドであることを確定した。また CcDOX1 にはヒドロペルオキシドを開裂する酵素活性はなく、ウシグソヒトヨタケ菌糸体ミクロソームに開裂酵素を新たに見出した。さらに *CcDOX1* 破壊株を用いて数種の菌食者の摂食行動を評価し、本研究で用いた菌食者は1-オクテン-3-オールを餌の探索因子としていることを明らかにした。

本論文に記載のこうした2つの研究成果は世界に先駆けた発見であり、それぞれ、Plant Physiology 誌、Journal of Biological Chemistry 誌に掲載されている。令和5年2月6日に実施した最終試験ではこれらの成果を丁寧に説明し、その後の質疑応答も適切であった。こうしたことを総合して本論文が博士学位を授与するに十分と判断した。