（学位第３号様式）

学 位 論 文 要 旨

氏　名	Mayumi Maeda
題　目	4-Vinylguaiacol production by *Aspergillus luchuensis* phenolic acid decarboxylase in *awamori* brewing. （泡盛醸造における *Aspergillus luchuensis* 由来フェノール酸脱炭酸酵素による 4-ビニルグアヤコール生産）

Awamori is a traditional distilled liquor in the Okinawa, Japan. It's made from steamed rice by the action of the black koji mold *Aspergillus luchuensis* and *awamori* yeast. *Awamori* aged in a tank for 3 years or longer is called "*kusu*". Vanillin, derived from ferulic acid (FA) in rice grains, is one of the characteristic flavors in *awamori kusu*. FA is released from the cell wall material in the rice grain by ferulic acid esterase produced by *A. luchuensis*. Decarboxylation of FA leads to the production of 4-vinylguaiacol (4-VG), which is converted to vanillin by natural oxidization. However, the mechanism underlying FA conversion to 4-VG has remained unknown in *awamori* brewing.

The genomic analysis of *A. luchuensis* revealed that the genes encoding the enzymes had sequence similarity to the enzymes phenylacrylic acid decarboxylase and ferulic acid decarboxylase of *Saccharomyces cerevisiae*, and phenolic acid decarboxylase (PAD) of bacteria and *Candida*. We hypothesized that *A. luchuensis* phenolic acid decarboxylase candidate gene (*alpad*), which is homology to bacterial and *Candida* PADs, is a major factor in 4-VG production in *awamori* brewing. In this study, first, the enzymatic characterization of recombinant AlPAD was done. Second, we analyzed the expression and function of AlPAD in *A. luchuensis*. Third, to understand the contribution of AlPAD to 4-VG production in *awamori* brewing, we created *alpad* disruptant (*Δalpad*) and compared the 4-VG productivity of *Δalpad* to that of the wild-type strain.

Recombinant AlPAD expressed as a homodimer, catalyzed the conversion of FA to 4-VG, displayed optimal catalytic activity at pH 5.7 and 40°C, and was stable up to 50°C. The cells cultured in rice bran or FA-containing medium showed FA to 4-VG bioconversion activity, and those activities correlated with the expression levels of AlPAD. Due to the absence of signal sequences in the *alpad* ORF, AlPAD should be localized to the cytosol. Therefore, *A. luchuensis* could take FA from the outside of the cell, convert it to 4-VG using AlPAD in the cytosol, and release the resulting 4-VG outside of the cell. The FA decarboxylation activity during the koji making increased, and the activity was correlated with the amount of AlPAD in the koji. The amount of 4-VG in the distillate of *moromi* prepared with the wild-type strain showed a significant increase that was proportional to the koji making time. In the *Δalpad* strain, the amount of 4-VG was very small and remained unchanged during the koji making. In an *awamori* brewing test using koji harvested 42-66 h after inoculation, the contribution of AlPAD to 4-VG production was in the range of 88-94 %. These results provide credible evidence that AlPAD is a major factor in 4-VG production during *awamori* brewing.