（学位第３号様式）

学　位　論　文　要　旨

氏　名	Jikian Tokashiki
題　目	Construction of protoplast-PEG transformation system for *Aspergillus luchuensis* and development of novel drug resistance marker gene （黒麹菌の形質転換系の構築と新規薬剤耐性マーカー遺伝子の開発）

Aspergillus oryzae is an important fungi in the production of commercial enzymes used for food processing, heterologous proteins, and fermented products such as sake, soy sauce, and miso (soybean paste). In addition, *Aspergillus luchuensis*, a black koji mold, is used for brewing traditional Japanese distilled spirits, such as awamori in the Okinawa islands and shochu in the Kyushu district.
A. luchuensis NBRC4314 recently underwent genome sequencing. We have not used the frequently-used protoplast–polyethylene glycol (PEG) method but have used agrobacterium-mediated transformation (AMT) for genetic engineering of this strain, because it was difficult to generate protoplasts using commercial cell wall lytic enzymes. In this study, I initially investigated the various conditions for protoplast formation in *A. luchuensis*. I found that *A. luchuensis* protoplasts could be generated using a minimal medium for the preculture with static conditions, and using Yatalase and α-1,3-glucanase as cell-wall lytic enzymes. These protoplasts could then be transformed with the protoplast–PEG method. Because α-1,3-glucanase was needed to form protoplasts in *A. luchuensis*, I investigated the role of the α-1,3-glucan synthase gene *agsE* in protoplast formation, one of five putative α-1,3-glucan synthase genes in *A. luchuensis* and a homolog of the major α-1,3-glucan synthase *agsB* in *Aspergillus nidulans*. I disrupted *agsE* in *A. luchuensis* (Δ*agsE*) with AMT and found that protoplast formation without using α-1,3-glucanase in Δ*agsE* was comparable with protoplast formation in *A. oryzae* with Yatalase. The Δ*agsE* protoplasts were also competent for transformation with the protoplast–PEG method. Hence, *agsE* appears to inhibit protoplast formation in *A. luchuensis*. Since two drug resistance markers that can be used in *A. luchuensis* have already been used for the Δ*agsE*, further transformation of this strain with drug resistance marker was limited. Therefore, I next addressed the development of a new drug resistance marker. Azole is a common and inexpensive antifungal drug that specifically inhibits fungi membrane biosynthesis. Utilizing this mechanism, various azole compounds such as itraconazole, voriconazole, and fluconazole have been used as agrochemicals for crop protection against fungal pathogens and as therapeutic drugs for opportunistic fungal infections. However, an azole-resistant gene that can be utilized as a selectable marker is not yet available. I focused on itraconazole, which has a comparatively low concentration of MIC among azole compounds and *cyp51A,* in which there are abundant data that single point mutations led itraconazole resistance from a clinical isolation in *Aspergillus fumigatus*. I examined whether *cyp51A* in *A. luchuensis*, which induces itraconazole resistance for *A. oryzae* and *A. luchuensis* transformants with only single point mutation. Consequently, using the cassette *A. luchuensis cyp51A* (*itrA*), which has a G52R mutation under the promoter for overexpression, I succeeded in producing itraconazole resistant strains in *A. oryzae* and *A. luchuensis*.