学 位 論 文 要 旨

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題　目	Breeding of *Monascus purpureus* strain KUPM5 exhibiting altered production ability of secondary metabolites, citrinin and monacolin K

Monascus purpureus is used in the production of red *koji*, other fermented foods, and supplements. *M. purpureus* produces a variety of secondary metabolites, including yellow, orange, and red pigments, monacolin K (MK), and citrinin. The red pigment is used as a food coloring compound. MK is a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase and is used as a drug to lower blood cholesterol levels. Citrinin, on the other hand, is a harmful compound, mycotoxins. The purpose of this study is to modify the production ability of the secondary metabolites MK and citrinin in the *M. purpureus* KUPM5 strain isolated in Thailand. The KUPM5 strain induced mutations using ultraviolet (UV) irradiation and NTG treatment with an alkylating agent to obtain mutants with lower citrinin production levels than the parent strain. Finally, two mutant strains KS301U and KS302U obtained by UV irradiation treatment and subsequent screening were selected. Both mutants produced 80% less citrinin in red *koji* than the parent strain KUPM5, and retained the ability to produce red pigment, which is a characteristic favorable to the food industry. Then, to improve the MK productivity of the parent strain KUPM5 strain, the strain was mutagenized by synchrotron light irradiation. Three mutants, SC01, SC02, and SC03, that showed higher MK production than strain KUPM5, were isolated from 936 colonies after screening. In particular, the mutant strain SC02 produced MK three times higher than strain KUPM5 did, and maintained the production capacity same to strain KUPM5, including red pigment, mycelium content, and α-amylase activity. Comparative genome analysis among strain KUPM5 and the mutant strains SC revealed that synchrotron light irradiation introduced mutations in approximately 90% of the total genes, including SNV (single nucleotide variants), MNV (single nucleotide variants), and Indel (Insertion and deletion) mutations. The frequencies of SNV substitution in the whole genome occupied 68.96% of all mutations, of which 92.38% were transversions and 7.62% were transitions. This study, therefore, proved the synchrotron light irradiation was highly efficient for the strain improvement of a filamentous fungus, *M. purpure*us, and provided insights into the properties of mutation in the fungus by this mutagen. Genome analysis of *M. purpure*us strain KUPM5 demonstrated that the total genome size of the strain KUPM5 was 24.47 Mb, with a GC content of 48.92% and the number of genes was predicted to be 9,270. The presence of the gene clusters involved in the biosynthesis of MK, red pigments and citrinin were found. MK-biosynthetic *mok* genes are present in three separated scaffolds, whereas the orthologous *mok* genes of *M. pilosus* strain BCRC38072 are clustered in a chromosome. It was revealed that there are differences in the structure of the *mok* gene cluster at the species level of the genus *Monascus*. The identification of various gene clusters for biosynthesis of secondary metabolites allow to discover new SM from *M. purpureus*. CRISPR/Cas9 approach was used to introduce the alteration of genome editing in the MK-hyperproducing strain SC02 to develop the strain that reduce the citrinin production. CRISPR/Cas9 system were designed to specifically inactivate the *citS*, the crucial gene in the citrinin biosynthetic gene cluster. Only mutant CR2 which had 1-base deletion, retained 48% of citrinin production, and the amounts of azaphilone pigments and MK were comparable to wt produced. This research have demonstrated that CRISPR/Cas9 can be developed as a tool for gene disruption in *M. purpureus*