Studies on barley miso flavor from koji fungus and breeding of koji fungus

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Koji fungus is a filamentous fungus used in many Japanese fermentation foods. Koji fungus produces starch decomposing enzymes in sake or shochu production, while it also produces protein and nucleic acid decomposing enzymes other than starch in miso and soy source production, and contribute to form good taste and flavor.

In this study, firstly, characteristic flavor of barley miso derived from koji fungus was analyzed and its relation with maturation was investigated. Analysis by sensory test showed that the characteristic flavor derived from koji fungus was significantly decreased as the maturation of barley miso progressed. While, in the comprehensive analysis result of volatile compounds using GC-MS, the amount of 1-octen-3-ol known as a characteristic flavor derived from koji fungus did not differ in various maturation degree of barley miso. On the other hand, most aldehyde compounds such as furfural were detected in barley miso with higher maturation degree. From these results, although the characteristic flavor derived

from koji in barley miso does not change, it was considered that other volatile compounds which increase during maturation could mask the koji like flavor.

Next, breeding of koji fungus by ion beam irradiation was studied in order to obtain various kind of koji fungus. Black koji fungus *Aspergillus luchuensis* RIB 2601 strain was subjected to ion beam irradiation and mutant U1 strain having high starch decomposing ability was obtained. In the U1 strain, the activity of amylolytic enzymes such as α-amylase, glucoamylase, α-glucosidase per cell weight was higher than that of the wild-type strain under all culture conditions, agar plate and rice koji. In the analysis by real-time RT-PCR, since there was no significant difference in transcription level of *amyA* (α-amylase) or *glaA* (glucoamylase) gene between wild-type and U1 strain, high enzyme production in U1 strain seemed to be due to adjustment after translation. Furthermore, strain U1 showed higher sensitivity to Calcofluor-white than the wild-type strain, and also contained higher amount of the *N*-acetylglucosamine in the cell wall compare to that of the wild-type strain. From these results, the structural defect of the cell wall in the U1 strain was considered. Also, RNA-seq analysis revealed that

transcriptional changes of at least 604 genes related to cell wall structure such as redox, transport, glucosamine-containing compound metabolism in U1 strain compare to the wild type strain. These results suggest that the possibility of obtaining high enzyme secretion capacity in U1 strain might be due to the structural change of the cell wall structure.