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Studies of Molecular Breeding Technique Using Genome Information on Edible Mushrooms

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Agrobacterium tumefaciens-mediated transformation(ATMT) of *Flammulina velutipes* was used to produce a diverse number of transformants to discover the functions of gene that is vital for its variation color, spore pattern and cellulolytic activity. Futhermore, the transformant pool will be used as a good genetic resource for studying gene functions. *Agrobacterium*-mediated transformation was conducted in order to generate intentional mutants of *F. velutipes* strain KACC42777. Then *Agrobacterium tumefaciens* AGL-1 harboring pBGgHg was transformed into *F. velutipes*. This method is use to determine the functional gene of *F. velutipes*. Inverse PCR was used to insert T-DNA into the tagged chromosomal DNA segments and conducting sequence analysis of the *F. velutipes*. But this experiment had trouble in diverse morphological mutants because of dikaryotic nature of mushroom. It needed to make monokaryotic fruiting varients which introduced genes of compatible mating types.

In this study, next generation sequencing data was generated from 28 strains of *Flammulina velutipes* with different phenotypes using Illumina Hiseq platform. Filtered short reads were initially aligned to the reference genome (KACC42780) to construct a SNP matrix. And then we built a phylogenetic tree based on the validated SNPs. The inferred tree represented that white- and brown- fruitbody forming strains were generally separated although three brown strains, 4103, 4028, and 4195, were grouped with white ones. This topological relationship was consistently reappeared even when we used randomly selected SNPs. Group I containing 4062, 4148, and 4195 strains and group II containing 4188, 4190, and 4194 strains formed early-divergent lineages with robust nodal supports, suggesting that they are independent groups from the members in main clades. To elucidate the distinction between white-fruitbody forming strains isolated from Korea and Japan, phylogenetic analysis was performed using their SNP data with group I members as outgroup. However, no significant genetic variation was noticed in this study.

A total of 28 strains of *Flammulina velutipes* were analyzed to identify the genomic regions responsible for producing white-fruiting body. NGS data was yielded by using Illumina Hiseq platform. Short reads were filtered by quality score and read length were mapped on the reference genome (KACC42780). Between the white- and brown fruitbody forming strains. There is a high possibility that SNPs can be detected among the white strains as homozygous because white phenotype is recessive in *F. velutipes*. Thus, we constructed SNP matrix within 8 white strains. SNPs discovered between mono3 and mono19, the parental monokaryotic strains of 4210 strain (white), were excluded from the candidate. If the genotypes of SNPs detected between white and brown strains were identical with those in mono3 and mono19 strains, they were included in candidate as a priority. As a result, if more than 5 candidates SNPs were localized in single gene, we regarded as they are possibly related to the white color. In *F. velutipes* genome, chr01, chr04, chr07,chr11 regions were identified to be associated with white fruitbody forming. White and Brown Fruitbody strains can be used as an identification marker for *F. veluipes*. We can develop some molecular markers to identify colored strains and discriminate national white varieties against Japanese ones.