Genome editing, non-GM breeding and post-genomics in edible mushrooms

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CRISPR/Cas9 is a popular technology for genome editing that can introduce a mutation at a target site on the chromosome through base-pairing between the target sequence and guide RNA (gRNA). By designing gRNA, CRISPR/Cas9 permits us to disrupt a specific gene in a living cell. Furthermore, using additional DNA fragment (donor DNA) containing sequence similarity around the target site, it can also introduce a designed mutation through homologous recombination. So, theoretically, it can edit the nucleotide sequence at the target site as we want. In breeding of crops, CRISPR/Cas9 provides great opportunities to control a gene with a desired phenotype. However, if foreign DNA from different species exist in the chromosome, the isolated cultivar is regarded as a genetically modified organism (GMO). For edible use, GMO is not favored and non-GM breeding is preferred for practical and commercial consumption.

In some counties such as USA and Japan, genome-edited cultivars without foreign DNA are recognized as a non-GM organism. In short, only genome-edited organisms with disrupted target gene are considered as non-GM and some of them are on the market. On 17th June 2026, EU decided to relax regulations and regard some genome-edited plant cultivars with a low level of alternation – changes that could also occur through conventional breeding as non-GMO. The new regulations are expected to come into force in mid-2028.

Pleurotus ostreatus (oyster mushroom) is a popular cultivated mushroom that belongs to basidiomycete and it can also use lignocellulosic materials as a substrate to grow. It is a white-rot fungus and can degrade recalcitrant polymer, lignin that cover polysaccharides such as cellulose and hemicellulose in plant cell wall. White-rot fungi play a key role in carbon recycling of the eco-system, since no other organisms can degrade lignin. In decays, not only as a conventional food, *P. ostreatus* has been target of intense research for pretreatment of lignocellulosic materials to be saccharified and fermented into bioethanol. Nowadays, as sustainable a material, mushroom mycelia are used to produce alternative leather,

composite for packing material or vegan meat.

With introduction of CRISPR/Cas9 into mushroom-forming fungi, such as *P. ostreatus* and *Lentinula edodes* (shiitake), now we can use it as a very powerful tool for molecular breeding of edible mushrooms. In this talk, I would like to briefly summarize current situation of genome editing in non-GM breeding of new cultivars among fruits, vegetables, and fish, in Japan. Furthermore, several CRISPR/Cas9 protocols for non-GM breeding in *P. ostreatus* will be presented as well as its use to solve a 'too much candidates' problem caused by post-genomics in our research life.