

Understanding of high protein secretion capability in *koji* mold by molecular and cellular biology techniques and its use as a cell factory

Katsuhiko Kitamoto

(The University of Tokyo, Department of Biotechnology, Professor)

【Outline of survey】

Koji mold (*Aspergillus oryzae*) is an important microorganism used to brew sake, *miso*, and soy sauce in Japan from of ancient times, and it is called as "Japan's National microorganism". *Koji* mold is also useful for the protein production such as enzymes because of its high protein secretion capacity of several grams in one-liter culture. Its safety is guaranteed due to its long history of use in the food manufacturing industry, and much attention is gained as an attractive host for the useful protein production used in various foods and medical treatments, etc. Moreover, the genome analysis project of *A. oryzae* was completed in 2005, bringing this organism into the genomic age. In this research we try to elucidate high capability of protein secretion with *koji* mold by molecular and cellular biology techniques and by making good use of genome information, for the purpose of using as a cell factory for useful protein production.

The quality control mechanism of the protein secretion in the endoplasmic reticula, visualization of the organelles (nucleus, endoplasmic reticulum, Golgi body, and vacuole, etc.) related to the protein secretion pathway, and the movement of the secretory proteins in the cell of *koji* mold will be analyzed. With the final result, *koji* mold that produces high levels of heterologous proteins will be bred.

【Expected results】

In general, filamentous fungi that are multi-cellular eukaryotic microorganism like *koji* mold have extremely high potential for protein secretion compared with yeast that is a unicellular eukaryotic microorganism. A little is known about the understanding at a molecular level of the protein secretion pathways in filamentous fungi, but this research will clarify the pathway. When heterologous proteins from higher eukaryotes are produced in *koji* mold, high productivity is not often obtained in general. By finding the solution to this bottleneck concerning useful protein production with *koji* mold, it is expected that a more refined cell factory system will be constructed.

【References by the principal researcher】

- Visualization of the endocytic pathway in the filamentous fungus *Aspergillus oryzae* using an EGFP-fused plasma membrane protein, Y. Higuchi et al., Biochem Biophys Res Commun., 340, 784-791 (2006)
- Genome sequencing and analysis of *Aspergillus oryzae*, M. Machida et al., Nature, 438, 1157-1161 (2005)
- Molecular Biology of the Koji Molds, K. Kitamoto, Adv. Appl. Microbiol., 51, 129-153 (2002)

【Term of project】 FY2006 - 2010

【Budget allocation】 20,700,000 yen

【Homepage address】

http://park.itc.u-tokyo.ac.jp/Lab_Microbiology/Englishfile/hyousi-E.html