Internship Proposition

Master 2 « Microbiology, Environment, Health »

1. Host Laboratory :

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PhD perspectives… :

☑ yes
o no

…with specific fundings :

o yes
☑ no

2. Internship Project: Title, description, approaches, references (maximum 2 pages) **:**

Title: Studies to Develop a Multiplex Genome Editing Method for Efficient Cell Engineering in the Filamentous Fungus *Aspergillus oryzae*

Backgrounds:

The filamentous fungus *Aspergillus oryzae* has been used in Japanese traditional fermentation. The fungus is also used in heterologous protein production due to its ability to secrete a large amount of enzymes into the medium (Nakajima *et al.*, 2006; Ito *et al.*, 2007).

Deletion of non-homologous end joining (NHEJ) genes such as *ku70* and *ligD* enables the highly efficient gene targeting (Maruyama and Kitamoto, 2008). Higher-level production of heterologous proteins was achieved by manipulating the genes involved in protein transport and degradation (Yoon *et al.*, 2010, 2013; Hoang *et al.*, 2015). By employing marker-recycling technique, the group of Dr. Maruyama has succeeded in generating the strain deleted with 10 protease genes, which has the ability to produce the higher amount of heterologous proteins (Yoon *et al.*, 2011). However, the multiple gene modifications are much laborious, since even one cycle of transformation of *A. oryzae* requires approximately 2 weeks.

Recently, the group of Dr. Maruyama established the CRISPR/Cas9 system as genome editing method (Katayama *et al.*, 2016). In this system, the Cas9 nuclease of *Streptococcus*

species is recruited to the target locus by the guide RNA. When the DNA strand cleaved by Cas9 is repaired, a mutation is introduced at the target chromosomal locus. The aim of this study is to develop a multiplex genome editing method for efficient multiple gene modifications in *A. oryzae*.

Experimental Plan

1. Establishment of multiplex genome editing in *A. oryzae*

First, multiple DNAs encoding for guide RNAs are inserted into the plasmid containing the gene for Cas9 nuclease. The generated plasmid with only a single selection marker is introduced into the *A. oryzae* strain. In this study, up to 4 genes (*e.g.* functionally redundant) will be targeted for the simultaneous mutagenesis by the CRISPR/Cas9 system.

2. Simultaneous genomic integration of multiple cellulolytic genes

Cellulosic biomass has been expected for the use for bioethanol production. In this study, to efficiently saccharify the cellulose, 3 types of cellulases (cellobiohydrolase, endoglucanase, and β-glucosidase) from the cellulolytic fungus *Aspergillus aculeatus* and a cellulose-loosing protein swollenin from the human-pathogenic fungus *Aspergillus fumigatus* (Chen *et al.*, 2010) will be produced by *A. oryzae*. These cellulolytic genes will be fused with the upstream/downstream flanking regions of the target locus for homologous recombination, and they will be simultaneously integrated into the different chromosomal loci by the multiplex genome editing method. The strain expressing 4 cellulolytic proteins is to be examined for the ability to efficiently degrade cellulose into glucose.

3. Simultaneous genomic integration of secondary metabolite biosynthetic genes

In recent years, *A. oryzae* has been used as a heterologous production host for secondary metabolites (*e.g.* Bailey *et al.*, 2016). As a secondary biosynthetic cluster consist of numerous genes, these genes were cloned into 3 or 4 separated plasmids with different selection markers. The *A. oryzae* strain NSAR1, which the group of Dr. Maruyama constructed to have 4 auxotrophic markers available for transformation (Jin *et al.*, 2004), was mainly used as the host. However, the ratio to obtain the strain expressing all the biosynthetic genes at the same time is not sufficiently high.

In this study, the biosynthetic genes for the secondary metabolite will be fused with the upstream/downstream flanking regions of the target locus for homologous recombination, and they will be simultaneously integrated into the different chromosomal loci by the multiplex genome editing method. A model secondary metabolite with the antibiotic activity will be selected (*e.g.* Bailey *et al.*, 2016). The efficiency of the multiplex genome editing is to be analyzed by seeing whether the transformant form a growth inhibition halo on the medium containing bacterial cells.

References:

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