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Dissertation Summary (approx. 800 words in English)

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（要　旨）(Summary)

Introduction

Basidiomycota and Ascomycota represent the major phyla of the fungal kingdom. Ascomycete yeasts, especially *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, are widely accepted as cellular biology model systems. Basidiomycota biology has been much less explored than Ascomycota, partly because there are no major Basidiomycota model organisms. A Basidiomycete yeast, *Naganishia liquefaciens*, was originally isolated from the deep-sea sediment in the Japan Trench. In this study, with the aim of establishing *N. liquefaciens* as a model organism, I develop advanced genetic tools that enable efficient gene targeting in this organism. Furthermore, I explore the genetic requirement for this homologous recombination (HR)-mediated gene targeting.

Experimental method

The gene targeting method employed electroporation with PCR products flanked by an 80 bp sequence homologous to the target. CRISPR-Cas9 system was developed for *N. liquefaciens* to increase gene targeting efficiency. Standard methodologies that have been developed for *S. cerevisiae* were used for manipulation of *N. liquefaciens*.

Results and Discussion

Establishment of a genetic transformation system for *Naganishia* yeast

Developing a genetic transformation system is the first step for the genetic manipulation of a given organism. Therefore, I established an electroporation-based transformation method to deliver DNA fragments into *N. liquefaciens* cells. I found the gene targeting efficiency depends on the length of homologous arms attached to the targeting construct. Gene targeting efficiency is around 25% with 1 kb of flanking homology while 80 bp homology reduced the targeting efficiency to approximately half of that observed with 1 kb homology. These data suggest that gene targeting in *N. liquefaciens* is not as efficient as in *S. cerevisiae*.

Construction of a CRISPR-Cas9 system for genome editing in *N. liquefaciens*

The overall low efficiency of gene targeting and transformation in *N. liquefaciens* prompted me to develop CRISPR-Cas9 system. In order to construct a transient Cas9 expression plasmid, I developed a single plasmid system bearing the Cas9 and the gRNA expression cassettes. Co-transformation of Cas9 expression plasmid with donor DNA achieved highly efficient gene targeting (>80%) even using short homology arms (80bp). This data clearly demonstrated that CRISPR-Cas9 expression stimulates gene targeting efficiency in *N. liquefaciens*.

Establishing gene complementation system for *Naganishia* yeast

Since a plasmid system is not available for gene complementation in *N.liquefaciens*, I developed a system that facilitates the integration of foreign DNA into *TRP2* locus by taking advantage of the efficient CRISPR-Cas9 system. To test the complementation system, *RAD51*, a central player in HR, was deleted and the resultant cells become sensitive to DNA damage in its absence due to defects in recombinational DNA repair. The damage sensitivity of the *rad51* mutant was complemented to the level of a wild-type strain by ectopically integrating WT *RAD51* gene into *TRP2* locus.

Genetic analysis for gene targeting in *Naganishia* yeast

DNA double-strand break repair, which includes non-homologous end-joining (NHEJ) and HR pathways, is considered to play important role in gene targeting. I found that blocking NHEJ pathway by deleting its

major component Ku70, almost completely inhibited inaccurate gene integration events. On the other hand, homology length controls the requirements for Rad51 and its mediator Rad52 in gene targeting. Importantly, gene targeting with short homology arms (80 bp) requires almost exclusively Rad52 while a *rad52* deletion mutant retains a relatively high efficiency with long homology arms (1 kb). By contrast, Rad51, which performs homology search and strand exchange in HR, plays a relatively minor role in gene targeting, regardless of the homology length. Interestingly, the absence of both Rad51 and Rad52 completely eliminated gene targeting even with the long homology arms.

Role of BRCA2 homolog, Brh2, in DNA repair of *N.liquefaciens*

Rad52 plays a predominant role in gene targeting in *N. liquefaciens*. However, the absence of Rad52 in *N. liquefaciens* does not cause severe sensitivity to DNA damaging sources such as ionizing radiation (IR) and UV, which is in stark contrast to the phenotypes of the *rad52* mutant in the Ascomycota yeasts *S. cerevisiae* and *S. pombe*. This observation suggests that *N. liquefaciens* may utilize a different mechanism in Rad51-dependent recombinational repair. I identified a homolog of human breast and ovarian tumor suppressor protein BRCA2, named Brh2 (BRCA2 homolog), in *N. liquefaciens*. Remarkably, a *brh2* deletion mutant exhibited very similar sensitivity to UV to the *rad51* deletion mutant and a brh2 rad51 double-deletion mutant showed very similar sensitivity to the *rad51* single deletion mutants. The result suggests that Brh2 functions in the Rad51-dependent DNA damage repair in *N. liquefaciens*.

Summary of findings

In this study, I developed advanced genetic tools that enable genetic modification of *N. liquefaciens*. The gene targeting method introduces PCR products flanked by an 80 bp sequence homologous to the target, combined with CRISPR-Cas9, routinely achieves 80% gene targeting efficiency. Importantly, I identified a human BRCA2 homolog, Brh2, in *N liquefaciens*. The *brh2* mutants were found to be sensitive to DNA damage to the same extent as the *rad51* mutants, indicating recombinational repair system in *N. liquefaciens* is BRCA2 (Brh2)-dependent.

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