Cloning and repression of *ku70* in *Pleurotus ostreatus*

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INTRODUCTION

White rot fungi belong to basidiomycetes and degrade plant cell wall lignin. They produced a family of extracellular ligninolytic enzymes, but function of each isozyme has not been not clearly elucidated yet, besides numerous enzymatic and biochemical analyses. Differential expression control in transcriptional level has been reported, however, a reverse-genetics technique would be more effective to clarify the role of each isozyme in biodegradation of lignin. To establish a reverse-genetic technique in basidiomycetes, it is required to develop an efficient gene targeting system. Gene targeting is to substitute a specific gene in the genome by homologous recombination (HR) in the living cell and will make it possible to evaluate the physiological role of an interested gene. HR is major repair pathways for DNA double strand breaks (DSB) in yeasts. In the HR pathway, the DSBs are repaired through regions homologous to each other. However it is generally considered that another repair pathway, non-homologous end joining (NHEJ) is predominant in somatic cells of multi cellular eukaryotes. In the NHEJ pathway, DSBs in chromosomes are repaired by joining two broken ends of DNA irrespective of their sequence homology. For the establishment of an efficient gene targeting system, it is necessary to repress the NHEJ pathway and to exaggerate the HR.

Recently, there were reports that deletion of an essential gene *ku70* for the NHEJ system increased greatly the efficiency of gene targeting in ascomycetes, *Neurospora crassa* [1] and *Aspergillus oryzae* [2]. In this study, the development of the gene targeting system by the repression of *ku70* was tried in basidiomycete *Pleurotus ostreatus*.

MATERIALS and METHODS

Degenerated primers for amplification of *P. ostreatus* gene fragment encoding for KU70 (*Poku70*) were designed with CODEHOP strategy [3], using highly conserved amino acid sequence among known KU70 proteins. Using these primers, a DNA fragment was amplified by PCR and cloned into pGEM-T vector. Then, a whole gene fragment was cloned using inverse PCR technique and full-length cDNA fragment was amplified by RACE methods and RT-PCR. Sequence analysis, phylogenic analysis and predicted molecular modeling of *Poku70* were done. To repress *Poku70*, we tried antisense RNA and RNAi methods. We constructed recombinant plasmids which contain a full-length or partical *Poku70* cDNA fragments in the antisence direction under the control of *P. ostreatus sdi1* promoter and terminator (p07uk series). Moreover, we constructed derivative plasmids which of p07uk series by inserting an intron from *P. ostreatus mnp2*. We also constructed RNAi plasmid which contains a partial cDNA fragment and intron followed by the same cDNA fragment in the antisense direction under the control of *P. ostreatus sdi1* promoter and terminator. Co-transformation of *P. ostreatus* by PEG/$CaCl_2$ protocol was carried out using antisense plasmids or RNAi plasmid as well as plasmids which contain a drug resistant marker gene.

RESULTS and DISCUSSION

The length of the cording regions of *Poku70* was 1947 bp which may code a peptide of 648 aa. Although the amino acid identities between *Po*KU70 and other KU70 proteins were not so high (20-30%), it was suggested that *Po*KU70 has highly conserved domains among KU70 proteins such as N-terminal α/β domain, β-barrel domain and SAP domain. Furthermore, the predicted molecular modeling of *Po*KU70 was extremely similar to 3D structure of human KU70.

We have not isolated recombinant *P. ostreatus* strains containing the antisense or RNAi sequences among the drug resistant transformants so far analyzed. Further experiments will be continued to repress *Poku70*.

REFERENCE

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