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Screening of Cell Cycle-Related Genes of *Pleurotus eryngii* Using Yeast Mutant Strains

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Temperature-sensitive yeast mutants were used to screen for cell cycle-related genes from *Pleurotus eryngii* genomic DNA. A mushroom genomic DNA library was established and each gene was screened for the ability to rescue seven *Saccharomyces cerevisiae* temperature-sensitive strains. Hundreds of yeast transformants were selected at restrictive temperatures over 30°C. Plasmids from the transformants that survived were isolated and transformed back into their host strains. The temperature sensitivity of the resulting transformants was tested from 30°C to 37°C. Ten DNA fragments from *P. eryngii* were able to rescue yeast temperature-sensitive strains, and their DNA sequences were determined.

KEYWORDS : Cell cycle, Genomic DNA library, *Pleurotus eryngii*, Temperature sensitive, Yeast

Cell division is a process by which a parent cell divides into two genetically identical daughter cells. The entire division process proceeds as a cycle of an S phase, where chromosome duplication occurs, and an M phase, where the two daughter cells separate. The two phases are separated by the gap phases G1 and G2. Mitosis is the process in which the duplicated chromosomes segregate into two daughter nuclei. It is followed immediately by cytokinesis, which divides the cellular components into roughly equal shares for each daughter cell. Mitosis and cytokinesis together define the mitotic (M) phase of the cell cycle [1].

Mitotic phase progression in *Saccharomyces cerevisiae* is controlled by several proteins, including the ser/thr protein kinases Cdc5 and Cdc15, the protein phosphatase Cdc14, and the GTPase Tem1 [2, 3]. The polo-like kinase Cdc5 plays multiple roles throughout the M phase. It promotes G2/M transition to allow initiation of the mitotic phase [4]. Cdc5 also initiates the separation of chromosomes in the middle of M phase [5], and it plays a role in cytokinesis as part of the mitotic exit network (MEN) [2]. Cdc5 promotes mitotic exit by phosphorylating and inhibiting the Bfa/Bub2 complex, which maintains Tem1 in an inactive state [6]. Inhibition of the Bfa1/Bub2 complex thus allows Tem1 activation by the guanine exchange factor Lte1, which converts inactivate Tem1-GDP to the active Tem1-GTP form. Tem1-GTP then binds to and activates Cdc15 and the MEN [7]. Only upon activation of the MEN can cytokinesis take place and cell division be completed. Therefore, Cdc5, Cdc15, and Tem1 are key modulators of the yeast M phase.

Pleurotus eryngii is one of the most cultivated edible mushrooms worldwide. This mushroom has been studied for years, but research has mostly focused on cultivation and bioremediation [8-10]. Basic molecular biology, such as cellular trafficking of molecules, the mechanism of cell division, and even detailed subcellular structures, etc. remain largely unexplored. In this respect, we have been interested in the mechanism of cell division in *P. eryngii*. In an effort to investigate genes involved in regulating the *P. eryngii* cell cycle, we developed a method to screen *P. eryngii* genes in temperature-sensitive mutants of *S. cerevisiae* for rescue of cell cycle defects at restrictive temperatures. The yeast cell division cycle is well-studied, and temperature-sensitive *S. cerevisiae* strains containing mutations of the key cell cycle modulators are readily available. As a result, this system is ideal for identifying *P. eryngii* orthologs of the yeast cell cycle regulators.

To generate the *P. eryngii* genomic DNA library, genomic DNA was extracted from a powder of the fruiting bodies of *P. eryngii* KNR2312 with QIAGEN i-genomic DNA extraction mini kits (INtRON Co., Seoul, Korea). The extracted genomic DNA was cut with the restriction enzyme *Sau*3A1, which generates a GATC overhang sequence on both ends of the DNA fragment. The restricted DNA fragments, ranging from 2~5 kb in length, were extracted from a 1% agarose gel and purified with a gel extraction kit (LaboPass Gel; Cosmo Gentech Co., Seoul, Korea). The extracted DNA fragments were ligated to the *Bam*HI site of a yeast centromeric vector, pRS315, which contains the *LEU2* gene as a selective marker. The optimal ratio between vector and DNA fragments was empirically determined to be 4 : 1. After overnight incubation of the reaction at 16°C, the ligation products were directly transformed into competent *Escherichia coli* DH5α cells. The number of *E. coli* clones was 2×10^5, and the average

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insert size was approximately 1 kb. The size of the *P. eryngii* genome is estimated to be approximately 40 Mb [11], so our library appears to cover the whole genome about 5-fold.

In order to screen the mushroom genomic DNA library in yeast *S. cerevisiae*, we obtained the temperature-sensitive mutant strains by kind donation from Dr. Kyung Lee at the US National Institutes of Health (NIH). The yeast strains in this study are as follows: KL1546 (wild-type), KL2395 (*cdc5-1*), KL2158 (*cdc15-2*), KL2391 (*tem1-3*), KL2392 (*tem1-3 Δbub2*), KL2412 (*tem1-3 Δbfa1*), KL2414 (*cdc5-1 Δbfa1*) and KL2398 (*cdc5-1 Δbub2*). All the mutants were derived from the *S. cerevisiae* W303 strain and show a mitotic arrest phenotype, meaning no growth at restrictive temperatures (above 33°C), because mitotic progress is dependent on the activities of the mutated gene products, which only function normally at permissive temperature (25°C).

Transformation of each yeast strain by the mushroom genomic DNA library was carried out as described previously [12]. In brief, the yeast cells were cultivated to OD_{600} = 0.6~0.8 in 100 mL of yeast extract-peptone-dextrose (YPD) culture media containing 1% yeast extract, 2% peptone, and 2% dextrose at 25°C with shaking at 100 rpm/min. Cells were harvested and suspended in 100 μL of 0.3 M lithium acetate (LiAc) in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). To this suspension, 3 μL of salmon sperm DNA and 15 μL of the mushroom genomic DNA library were added, and the mixture was allowed to stand at room temperature for 30 min. Afterwards, 300 μL of polyethylene glycol 4000 solution (40% in LiAc-TE buffer) was added to the mixture and then incubated at 42°C for 15 min. The suspensions were spun twice to remove all the liquid. Finally, the cells were suspended in a small amount of SOS medium (10 mL of 2 M sorbitol, 1.34 mL of 5 × YPD, 0.13 mL of 1 M $CaCl_2$, 8.53 mL of water).

Primary selection of transformed colonies was carried out on a leucine dropout minimal medium plate (SD-Leu) containing 6.7 g/L of Yeast Nitrogen Base (BIO101 Co., Vista, CA, USA), 0.72 g/L of CSM-Leu (BIO101 Co.), and 2% glucose. To screen for yeast cells that overcame the temperature-sensitive phenotype through the introduced mushroom gene, colonies on the SD-Leu plate were transferred to four YPD plates by a replica plating method. The YPD plates were then incubated at a permissive temperature (25°C) and several restrictive temperatures (30°C, 33°C, and 37°C). All of the cells grew as normal at 25°C, which is suitable for yeast growth. Growth at 30°C was not much different from growth at 25°C. The temperature 33°C seemed to be restrictive to some of the transformants because most of them showed only weak growth at this temperature. Almost all the transformants were unable to grow at 37°C (Fig. 1). We predicted that the transformants that could survive at higher temperatures were rescued from temperature sensi-

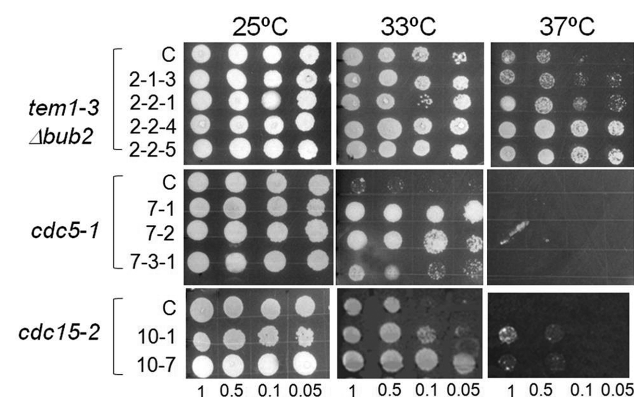

Fig. 1. Suppression of temperature-sensitive phenotypes of *Saccharomyces cerevisiae* mutant strains by *Pleurotus eryngii* (*P. eryngii*) genomic DNA. Cell cycle-defective yeast host strains (*tem1-3Δbub2*, *cdc5-1*, and *cdc15-2*) were transformed with plasmid clones containing *P. eryngii* mushroom DNA. The transformants were serially diluted with liquid medium (1 ×, 0.5 ×, 0.1 ×, 0.05 ×) and spotted onto 3 yeast extract-peptone-dextrose plates. The plates were incubated at the permissive (25°C) or restrictive (33°C and 37°C) temperatures. The clone numbers are listed next to each host strain name.

Table 1. Selected *Pleurotus eryngii* genomic DNA clones and their homologous proteins

Host genotype	Clone No.	Protein function	Gene ID	Organism
tem1-3Δbub2	2-1-3	Dehydrogenase E1 and transketolase domain containing 1 (DHTKD1)	AAH07955	*Homo sapiens*
	2-2-1	Predicted protein	EER31102	*Candida tropicalis*
	2-2-4	Predicted protein	EDR04975	*Laccaria bicolor*
	2-2-5	Gamma tubulin interacting protein	EDO98538	*Chlamydomonas reinhardtii*
	2-2-7	Putative urea carboxylase	EEQ19015	*Aspergillus nidulans*
cdc5-1	7-1	Unnamed protein product	BAG64005	*Homo sapiens*
	7-2	DC1 domain-containing protein	CAB51185	*Arabidopsis thaliana*
	7-3-1	DNA polymerase epsilon catalytic subunit	EEE30018	*Toxoplasma gondii*
cdc15-2	10-1	Hedgehog/intein hint domain protein	EEQ19015	*Yersinia intermedia*
	10-7	Hypothetical protein	EEB92681	*Moniliophthora perniciosa*

tivity by the induced genes from mushroom genomic DNA. From this initial screening, a total of 129 transformants that formed colonies at 33°C and 37°C were selected for further screening.

In order to confirm whether survival at the restrictive temperature was due to *P. eryngii* DNA, we recovered 25 plasmid DNAs from the yeast transformants that exhibited the strongest growth at the restrictive temperature and determined their DNA sequences. The recovered plasmids were also separately transformed back into their original hosts, and their effects on host growth were examined in serially diluted liquid cultures. Among the 25 plasmids selected for further screening, 10 plasmids showed the best growth at 33°C or 37°C (Fig. 1). Analysis of the determined sequence revealed that the *P. eryngii* sequences contained in the plasmids mostly carried DNA fragments with unknown functions (Table 1). The three transformants of the *tem1-3Δbub2* strain that carry the plasmids 2-2-4, 2-2-5 and 2-2-7 had good growth at 37°C, whereas the control host cells could hardly survive (Fig. 1). Interestingly, sequences from clone 2-2-5 showed some homology with a γ-tubulin interacting protein of *Chlamydomonas reinhardtii* (Fig. 2A). Tubulin disassembly is an important process during mitotic exit [13], suggesting a possible mitotic role of the mushroom DNA contained in clone 2-2-5. Clone 2-2-7 contained a sequence with high homology to the putative urea carboxylase of *Aspergillus nidulans*, but its role in the cell division is largely unknown (Fig. 2C). We were also able to isolate 3 and 2 more mushroom library plasmids that suppressed *cdc5-1* and *cdc15-2* temperature-sensitive growth arrest, respectively. BLAST analysis of the genes predicted a few homologous proteins with unknown functions. Only clone 10-1, which suppressed temperature sensitivity of the *cdc15-2* strain, appeared to show some homology with the hedgehog/intein hint domain protein of *Yersinia intermedia* (Fig. 2B). Hedgehog has been reported to upregulate cell cycle proteins [14]. For further information, more rigorous analyses of these identified DNA sequences are required in both *P. eryngii* and *S. cerevisiae*.

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A
Clone 2-2-5  ASLVYYKATLSVLLLAIEGRARQVTSKNAAAK-RVQDRGWGILAMA
             A++V  +A  + L  A+EG AR++ ++ AAA+ RV   GWG+ A A
ED098538     ANMVGLRAVAARLEEAVEGGARKLQTRTAAARQRVARGGWGVQAPA

Clone 2-2-5  WAGIYTRIGSCAAEHSTSTAGGPFISGMRRPRLLNLGPSG
             W   YTR  SCA   S  TA    ++ + R  LL  G  G
ED098538     WQEFYTRAASCADLDSLITAHEEHLAKLLRKALLEGGGGG

B
Clone 10-1   PRPAGGTFPGCARRALGLVEVAFAGLECRAVDDGFGARGVLLTWEVPGENGQIKLKQGTA
             P  A GTFP   ++    V + +   +       +GA  ++ T   P      KL Q  +
EEQ19015     PIQADGTFPASLKQTRYPVNIIYTFSQTVNNQTVYGAE-IVTTQSYP------KLIQNDS

Clone 10-1   PI---KTAFI---VARGHVQCSYYAKFNGNVFAQVKSHVTVRSATQTITISWFPTVDCQF
             P    K   I   +AR    C Y+  F+GNV   +K  VT           +F  +D
EEQ19015     PADLNKNNLIKICLARKENDCDYFNSFDGNVTVPIKGSVT-----------YFGNIDLNN

Clone 10-1   SGPVISHAQASTAILALRRRGKAARSPARGCYFFKELK
               P+ ++   ++  L   R+G    +PA    FF + K
EEQ19015     GKPINAY---NSIYLVRERQGGDPITPASNFNFFDDAK

C
Clone 2-2-7  LLVANRGEIAVRILTTARKLGLQTISVYSPSDATSLHVGLADEAIPLADYRHDKRKPTKG
             LL+ANRGEIAVR+L TA+KL ++TI+VY+  DA S HV LADEAI L+
CBF88588     LLIANRGEIAVRVLKTAKKLNIRTIAVYTEPDAASTHVHLADEAILLSG-----------

Clone 2-2-7  TLNEDITASPTEGEDGVPESQLYLDAHLLLSICKELGATLVHPGYGFLAENAGFIRLFTE
                              P S+ Y+D   ++ I K  GA  + PGYGFL+EN+ F R
CBF88588     -----------------PPSKAYIDGDQIIDIAKRKGADAIIPGYGFLSENSNFARDVAS

Clone 2-2-7  TGITVLAPSADVVELMGAKHAAREIARKVGVRVCPGSGDEPSSIEMDNVDGLTTSLDAAI
              G+  + PS + +E  G KH ARE+A K GV + PGS             GL TS D A+
CBF88588     AGLAFVGPSPESIEAFGLKHTARELATKAGVPIVPGS------------QGLVTSEDEAV

Clone 2-2-7  ELGKRVGFPILLK
             ++ + +GFP++LK
CBF88588     KIAQSLGFPVMLK
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Fig. 2. BLAST analysis of three mushroom DNA sequences that suppressed yeast temperature-sensitive phenotypes. A, Clones 2-2-5 and C, 2-2-7 were selected as suppressors of the yeast *tem1-3Δbub2* strain. B, Clone 10-1 was a suppressor of the yeast cdc*15-2* strain.

Agriculture and Forestry, Republic of Korea.

References

1. Nurse P. Cyclin dependent kinases and cell cycle control (nobel lecture). Chembiochem 2002;3:596-603.
2. Rahal R, Amon A. The Polo-like kinase Cdc5 interacts with FEAR network components and Cdc14. Cell Cycle 2008; 7:3262-72.
3. König C, Maekawa H, Schiebel E. Mutual regulation of cyclin-dependent kinase and the mitotic exit network. J Cell Biol 2010;188:351-68.
4. Darieva Z, Bulmer R, Pic-Taylor A, Doris KS, Geymonat M, Sedgwick SG, et al. Polo kinase controls cell-cycle-dependent transcription by targeting a coactivator protein. Nature 2006;444:494-8.
5. Visintin R, Stegmeier F, Amon A. The role of the polo kinase Cdc5 in controlling Cdc14 localization. Mol Biol Cell 2003; 14:4486-98.
6. Geymonat M, Spanos A, Walker PA, Johnston LH, Sedgwick SG. In vitro regulation of budding yeast Bfa1/Bub2 GAP activity by Cdc5. J Biol Chem 2003;278:14591-4.
7. Ro HS, Song S, Lee KS. Bfa1 can regulate Tem1 function independently of Bub2 in the mitotic exit network of *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 2002;99:5436-41.
8. Choi UK, Bajpai VK, Lee NH. Influence of calcinated starfish powder on growth, yield, spawn run and primordial germination of king oyster mushroom (*Pleurotus eryngii*). Food Chem Toxicol 2009;47:2830-3.
9. Rodriguez Estrada AE, Jimenez-Gasco Mdel M, Royse DJ. Improvement of yield of *Pleurotus eryngii* var. *eryngii* by substrate supplementation and use of a casing overlay. Bioresour Technol 2009;100:5270-6.
10. Stajiæ M, Vukojeviæ J, Duletiæ-Lauseviæ S. Biology of Pleurotus eryngii and role in biotechnological processes: a review. Crit Rev Biotechnol 2009;29:55-66.
11. Kullman B, Tamm H, Kullman K. Fungal genome size database 2005 [Internet]. Available from: http://www.zbi.ee/fungal-genomesize.
12. Gietz RD, Woods RA. Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. Methods Enzymol 2002;350:87-96.
13. Monje-Casas F, Amon A. Cell polarity determinants establish asymmetry in MEN signaling. Dev Cell 2009;16:132-45.
14. Katoh Y, Katoh M. Hedgehog target genes: mechanisms of carcinogenesis induced by aberrant hedgehog signaling activation. Curr Mol Med 2009;9:873-86.