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A Deletion in Fungal *Ras* Promoter in Two Korean Strains of Oak Mushroom (*Lentinula edodes*)

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This study unexpectedly detected a deletion in the promoter region of *ras* gene in two Korean strains of oak mushrooms, *Lentinula edodes* (Berk.). Sequencing of the promoter regions revealed that one type consisting of two strains had a 113 bp deletion in the region. The *ras* promoter region of Korean strains differed by 16 bases from that of the Japanese strains. Between the two types of Korean strains, except for the deleted portion, only a single site appeared to be different.

Keywords : deletion, *Lentinula edodes*, oak mushroom, *ras* promoter.

Oak mushroom, *Lentinula edodes* (Berk.), belongs to the family Tricholomataceae. This edible mushroom grows on the stems of oak tree species (*Quercus spp*) in the wild. However, for commercial production, it is being cultivated using sawdust and other artificial media. Although a number of strains have been known in the market, many more are being developed in Korea, Japan, and China.

Ras genes are present in all organisms, and are known to play a role in growth and development (Shin and Yun 1996). In mammalian cells, they are involved in the genesis of a wide variety of tumors (Ross, 1996). Although there have been some speculations that *ras* genes might be involved in fruiting body formation in some fungi, there has been no direct evidence to support this theory (Hori et al., 1991). The promoter region of the *ras* gene in *L. edodes* was physically examined by Kajiwara and Shishido (1992). However, its expression pattern in *L. edodes* has not been established yet.

This study attempted to develop T-DNA vectors for mushroom transformation. As selectable markers, antibiotics resistance genes (*nptII* and *hpt*) under the control of nopaline synthase promoter, known as a constituitive plant expression promoter (An et al., 1986), were used. However, no transformants were obtained from those vectors. It was speculated that the plant promoter was not working in the mushroom system. Therefore, the use of *ras* promoter was decided upon since it has already been characterized in the same species (Kajiwara and Shishido, 1992). In the process, a deletion in the *ras* promoter region in some of the Korean strains was unexpectedly found. This paper reports the nucleotide difference in the deletion mutants of *L. edodes*.

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Materials and Methods

Eight different strains of *L. edodes* with various shapes, habitats, and growing patterns were used in this study. The characteristics of the strains are described in Table 1. In addition, two uncharacterized strains (Pyogo 16 and 164) that had been maintained in culture were included for PCR pattern comparison.

DNA extraction. DNA was extracted from the mycelia of *L. edodes* by alkaline lysis method used for plant DNA extraction with a slight modification (Junghans and Metzlaff, 1980). The modification was a prolonged incubation (3 h) in lysis buffer at 65°C.

PCR amplification and cloning of RAS promoter region. The primer sequence for the amplification of *ras* promoter was designed based on the promoter sequence of Japanese strain FMC2 (Kajiwara and Shishido, 1992). The primers include LE 1: 5'-AAG CTT CGC ATA GCG GGA TCA TAT G-3' and LE 2: 5'-GGA TCC AGA AAC AGT CGG CTC CTC A-3'. Each reaction mixture contained 0.4 μM primers, 200 nM dNTPs, 0.2 ng template DNA, 2 U of Taq DNA polymerase and PCR buffer

Table 1. Characteristics of *Lentinula edodes* strains tested

Strain	Temperature*	Suitable medium for cultivation
Sanlim1	Low	Log
Sanlim2	High	Log
Sanlim3	Low	Log
Sanlim4	High	Log
Sanlim5	High	Sawdust
Sanlim6	High	Sawdust
Sanlim7	High	Log
Sanlim8	Medium	Log

*Optimal temperature range for the formation of fruiting bodies. Low: 5-15°C, Medium: 10-20°C, High: 15-25°C

provided by the manufacturer (Promega Co., USA). The PCR started for 2 minutes at 95°C and continued with 38 cycles of 94°C (20 seconds), 55°C (40 seconds), and 72°C (2 minutes). The amplification product was run on 1.2% agarose gel, eluted from the gel, and cloned into a PCR cloning vector (pGEM-T: Promega Co., USA).

Analysis of the sequence. The sequences of the cloned fragments were determined by ABI 377 genetic analyzer and analyzed by the programs provided by the Baylor College of Medicine Search Launcher (http://Searchlauncher.bcm.tmc.edu/). The *ras* promoter sequences of both Sanlims 3 and 4 were registered in the GenBank (NCBI) and, thus, could be accessed by the numbers AF479615 and 479616, respectively.

Results and Discussion

Difference in size of amplification products. Fig. 1 shows the PCR products of *ras* promoter region using the primers LE 1 and 2. Two strains (Sanlim 3 and 6) yielded smaller band (ca. 600bp) than did other strains (ca. 700bp). Based on the amplification products, 8 out of 10 Korean strains tested appeared to have the same size of promoter with that of the Japanese strain FMC2 reported by Kajiwara and Shishido (1992). However, Table 1 shows that the two strains, Sanlim 3 and Sanlim 4, were not as close to each other as to other strains with regards to cultivation temperature and media, although both had smaller *ras* promoters.

Sequence comparison. The nucleotide sequences of the *ras* promoter in the two different types (Sanlim 3 and Sanlim 4) were compared with those of the Japanese strain registered in the GenBank (NCBI). While Sanlim 4 appeared to have similar size to that of the Japanese strain, Sanlim 3 had a deletion in the *ras* promoter region. Fig. 2 shows the alignment of three sequences based on nucleotide homology. There was a 113bp deletion in the region in the strain Sanlim 3. Other than the deleted portion, only one base was different between the two Korean strains. The Korean strain Sanlim 4 was very similar to Japanese strain FMC2 in the promoter region. However, they differred by 16 bases from each other. This suggests that although the two types of Korean strains differ in the size of amplification products due to the deletion, they appeared to be closer to each other than to the Japanese variety (Fig. 3). At present, the significance of the deletion in the promoter region in some strains of this mushroom is not known yet. Since the strains showing the deletion are quite different from each other in their growth habit and culture, such deletion may not be significant at all in determining the characteristics described in Table 1. Although the promoter region has been structurally characterized by Hori et al. (1991) and Kajiwara and Shishido (1992), neither the expression pattern nor the regulation mechanism have been known. Chimeric genes

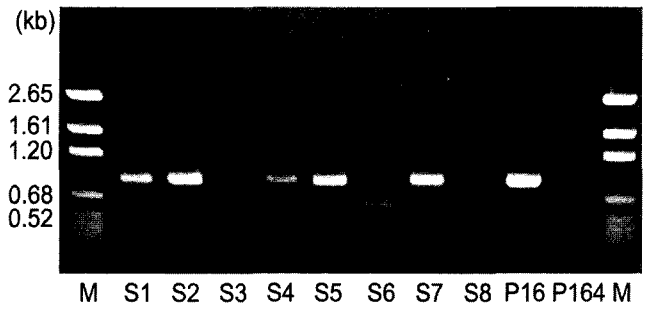

Fig. 1. PCR amplification products of *ras* promoter region in 10 different strains of *Lentinula edodes*. Lane M: pGEM DNA molecular marker, Lanes 1 to 10 are Sanlim 1, 2, 3, 4, 5, 6, 7, 8, Poygo 16 and 164.

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FMC2 CTTCGCATAG CGGGATCATA TGAATCATTT CCAGATGGTT GACGTTCATA TGT-AAAGCG
San3 CTTCGCATAG CGGGATCATA TGAATCATTT CCAGATGGTT GACGTTCATA TGTTAAAGCG
San4 CTTCGCATAG CGGGATCATA TGAATCATTT CCAGATGGTT GACGTTCATA TGTTAAAGCG

FMC2 CTATTCAAAG GCACGCACAG ATACCGAACA CG-ACTACGT ATGTATAT-A ACTGTCATTT
San3 CTATTCAAAG ---------- ---------- ---------- ---------- ----------
San4 CTATTCAAAG GCACGCACAG ATACCGAACA CGCACTACGT ATGTATATGA ACTATCATTT

FMC2 AGTTTTCACT GAACAAATTG ACAGCACTAT CACATACCTT ACACCGACCA AGAAAGAAGA
San3 ---------- ---------- ---------- ---------- ---------- ----------
San4 AGTTTTCACT AAACAGATTA ACAGCACTAT CACATACCTT ACACCAACCA AGAAAGAAGA

FMC2 TCTATTTTTC TCAAGTCCTT GACTTCTCTA CCTAGCGATC CTCACACCTT TAACTTCCCG
San3 --TATTTTTC TCAAGTCCT- GACTTCTCTA CCTAGCGATC CTCACACCTC TAACTTCCCG
San4 TCTATTTTTC TCAAGTCCT- GACTTCTCTA CCTAGCGATC CTCACACCTC TAACTTCCCG

FMC2 TGTT-CCTTG AGCATGATTC AACAAGTCGA AACCTTCTCG CGAACGACGA GGATGTGCAG
San3 TGTTTCCTTG AGCATGATTC AACAAGTCGA A-CCTTCTCG CGAACGACGA GGATGTGCAG
San4 TGTTTCCTTG AGCATGATTC AACAAGTCGA A-CCTTCTCG CGAACGACGA GGATGTGCAG

FMC2 CGTAAATCAC CAACCATCAA GAGGGGTTCA ATTTAGATGA GAGATGCATC GATGAGTGAT
San3 CGTAAATCAC CAACCATCAA GAGGGGTTCA ATTTAGATGA GAGATGCATC GATGAGTGAT
San4 CGTAAATCAC CAACCATCAA GAGGGGTTCA ATTTAGATGA GAGATGCATC GATGAGTGAT

FMC2 ATAACATGTC GAGTTGGAAA AGGCGTGGCT TGAGACAACA CGGACACAGC TTCTTGAACC
San3 ATAACATGTC GAGTTGGAAA AGGCGTGGCT TGAGACAACA GCGACA--GC TTCTTGAACC
San4 ATAACATGTC GAGTTGGAAA AGGCGTGGCT TGAGACAACA GCGACA--GC TTCTTGAACC

FMC2 AGAATAGGGA GCTTCGATGC AGCTGCGCAC AGCCCACACC CCCAGCCAGT GATACTATGT
San3 AGAATAGGGA GCTTCGATGC AGCTGCGCAC AGCCCACACC CCCAGCCAGT GATACTATGT
San4 AGAATAGGGA GCTTCGATGC AGCTGCGCAC AGCCCACACC CCCAGCCAGT GATACTATGT
                                                                    *
FMC2 ACTGTGATAC TATAAAACCT GTATAAAACC ATTTTGCCGT GCTCGTCGTT GATTAAGATC
San3 ACTGTGATAC TATAAAACCT GTATAAAACC ATTTTGCCGT GCTCGTCGTT GATTGAGATC
San4 ACTGTGATAC TATAAAACCT GTATAAAACC ATTTTGCCGT GCTCGTCGTT GATTAAGATC

FMC2 TTGGTCCTTG GCAAAATAAA ATAAACCTCG TGCCCGAACA CGTGAATCCA ACCCTCTTTT
San3 TTGGTCCTTG GCAAAATAAA ATAAACCTCG TGCCCGAACA CGTGAATCCA ACCCTCTTTT
San4 TTGGTCCTTG GCAAAATAAA ATAAACCTCG TGCCCGAACA CGTGAATCCA ACCCTCTTTT

FMC2 GGATCTCGGA ATTCAACGCC TTTTTAGGGT TCTACCCGCT TTTCCCATCT TCCCCTTTAT
San3 GGATCTCGGA ATTCAACGCC TTTTTAGGGT TCTACCCGCT TTTCCCATCT TCCCCTTTAT
San4 GGATCTCGGA ATTCAACGCC TTTTTAGGGT TCTACCCGCT TTTCCCATCT TCCCCTTTAT

FMC2 TCTTTGGTTG TCCTTCTTCT TCTTCTTTTC CAAAAGACTT GAGGAGCCGA CTGTTTCT
San3 TCTTTGGTTG TCCTTCTTCT TCTTCTTTTC CAAAAGACTT GAGGAGCCGA CTGTTTCT
San4 TCTTTGGTTG TCCTTCTTCT TCTTCTTTTC CAAAAGACTT GAGGAGCCGA CTGTTTCT
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Fig. 2. Nucleotide sequence alignment of the *ras* gene promoter region with Japanese strain FMC2 (NCBI Accession D10987 D01208) and two Korean strains Sanlim 3 and 4. Each dot represents the position showing different nucleotide between Japanese and Korean strains. Asterisk represents the position showing difference between the two Korean strains.

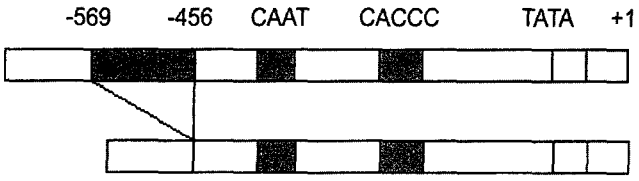

Fig. 3. Deleted portion of upstream region of *ras* promoter in the strain Sanlim 3 (bottom) of *Lentinula edodes* confirmed by sequencing. The top is the *ras* promoter region of the strain Sanlim 4. +1 represents a major transcriptional initiation site.

are constructed by attaching the *ras* promoters to GUS gene. If transgenic mushrooms carrying the chimeric gene could be obtained, changes in their expression pattern during their life cycle could be detected.

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