

# Commercial strain typing of *Lentinula edodes* with inter-retrotransposon amplified polymorphism-PCR method

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*Lentinula edodes* (Berk.) Pegler has many commercial strains both morphologically and physiologically similar to each other. At present, detection of polymorphism in rDNA-IGS region (Babasaki, 2006) and/or RAPD marker (Zhang and Molina, 1995) is generally used for strain typing of *L. edodes*. However, it is rather time-and-cost consuming. Inter-retrotransposon amplified polymorphism (IRAP)-PCR method mainly used for horticultural crops takes less time and lower in cost in strain typing (Kalendar et al, 1999). In this study, we designed IRAP primers for *L. edodes* and verified their strain typing efficiency.

## ※Methods※

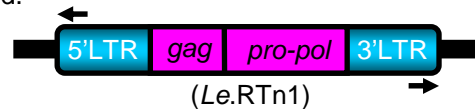
### <DNA extraction>

Either fungal cultures on PDA or fungal tissues of fruit bodies were cut into approximately 4 x 4 x 4 mm. Total DNA of each samples were extracted by DNeasy Plant Mini Kit (QIAGEN).

### <Primer design>

Based on LTR (Long Terminal Repeat) sequence in retrotransposon (*Le.RTn1*) of *L. edodes*, one set of primers amplifying the regions between retrotransposons were designed.

**AGGGGCTGCGTACACTTTATGAC** and  
**TAGTCACTCCGCCTCTACCGCTTCG**



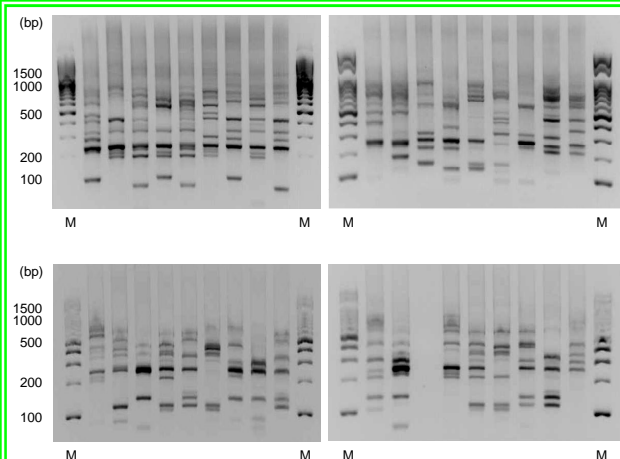
### <PCR>

For PCR, IRAP primer set and Pfu-X polymerase.(greiner) were performed. Performing thermal cycling conditions are as follows: **Precycle: 94 °C for 4 min. Cycling: 35cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 2 min. External elongation: 72°C for 10 min. Hold: 7 °C forever.**

### <Electrophoresis>

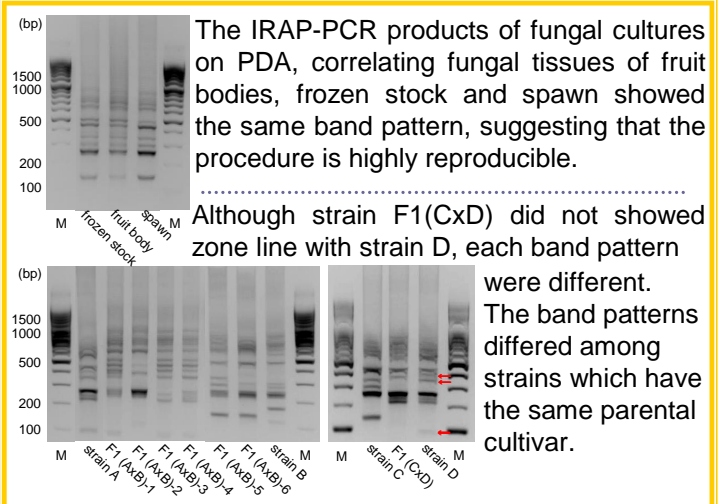
Ampricons were electrophored for 50 min at 100 V on 1.7 % agarose gel with GelRed (Biotium) and evaluated under UV irradiation.

## ※Results and discussion※



The products obtained by IRAP-PCR were determined using mini-gel electrophoresis system. The band patterns were different among strains. The detected bands were bright and clear.

When cluster analysis were performed with detected band patterns, there were no clusters by cognate strains. It is considered for the reason that IRAP-PCR do not participate in difference arisen from nucleotide substitution.



## CONCLUSION

IRAP-PCR with short ranged (ca. 1 kb) electrophoresis is a time-efficient and practical strain typing method of *L. edodes*.

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