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Detection of pathogen associated with root rot in infected ginseng using novel microsatellite markers

Seung Ho Lee*, Chi Eun Hong and Kyong Hwan Bang

Ginseng Research Division, Department of Herbal Crop Reserch,
National Institute of Horticultural and Herbal Science, RDA, Eumseong 27709, Korea

책임운영기관 국립원예특작과학원 National Institute of Horticultural and Herbal Science

Ⅰ. Abstract

Background : Ginseng (*Panax ginseng* C.A. Meyer) is one of the most important medicinal plants in Korea, but its yields are often reduced by a variety of root pathogens. The root rot of ginseng is a destructive soil-borne disease caused by *Paraphoma radicina*, *Plectosphaerella cucumerina*.

Methods and Results : To explore detection marker at novel microsatellite loci, we developed 30 markers after identifying repeat sequences in the genome of *P. radicina* and *P. cucumerina* with simple sequence repeat (SSR) mitifs analysis, respectively. Based on the PCR band patterns by electrophoresis, these novel microsatellite primers could be classified into other ginseng pathogens (*Cylindrocarpon* sp., *Fusarium* sp., *Alternaria* sp., etc). One of Real time PCR assay was developed for rapid detection and quantification of *P. radicina* and *P. cucumerina* in diseased ginseng roots and artificially inoculated soil.

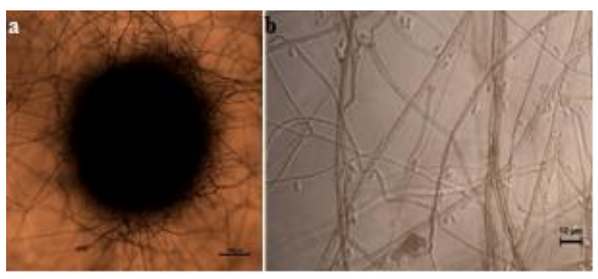
Conclusion : The approach outlined here could be further utilized as a rapid and reliable tool for the diagnosis and monitoring of the root rot of ginseng

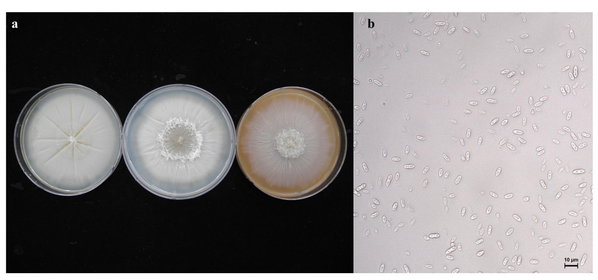
Ⅱ. Material & Method

- **Ginseng root rot related pathogens : *P. radicina, P. cucumerina*.**
- **Genome analysis**
  - **Illumina & Nanopore sequencing**
  - **Genome assembly & functional annotation**
  - **Microsatellite : Type of motif**
- **Designs of PCR marker**

Ⅲ. Results

New pathogens associated ginseng root rot

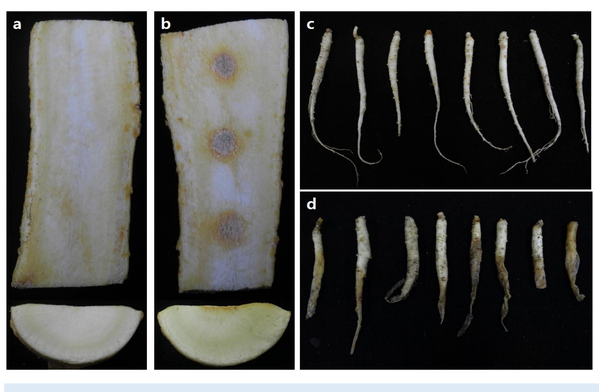




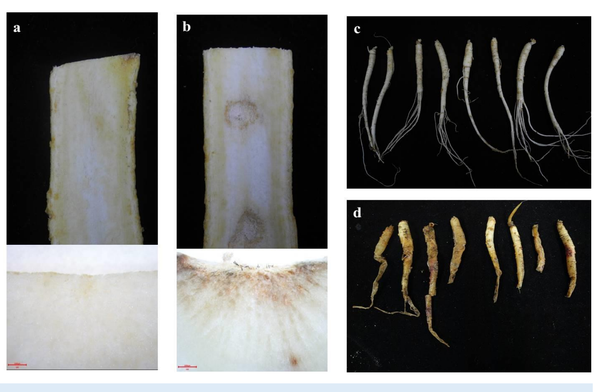
Fungi	*P. radicina*	*P. cucumerina*
Setose pycnidium	321 ~ 461 um	-
Conidia	4.5 ~ 1.7 um	8.1 X 2.8 um

Pathogenicity

<P. radicina>



<P. cucumerina>



- **Pathogens of Ginseng root rot**
  - **Major : *Fusrium solani* (Fs) > *Ilyonectria robusta* (Ir) > *I. mors-panacis* (Im)**
  - **Minor : *P. radicina* (Pr), *P. cucumerina* (Pc)**
  - **Pathogenicity : Im > Ir ≥ Fs, Pr, Pc**

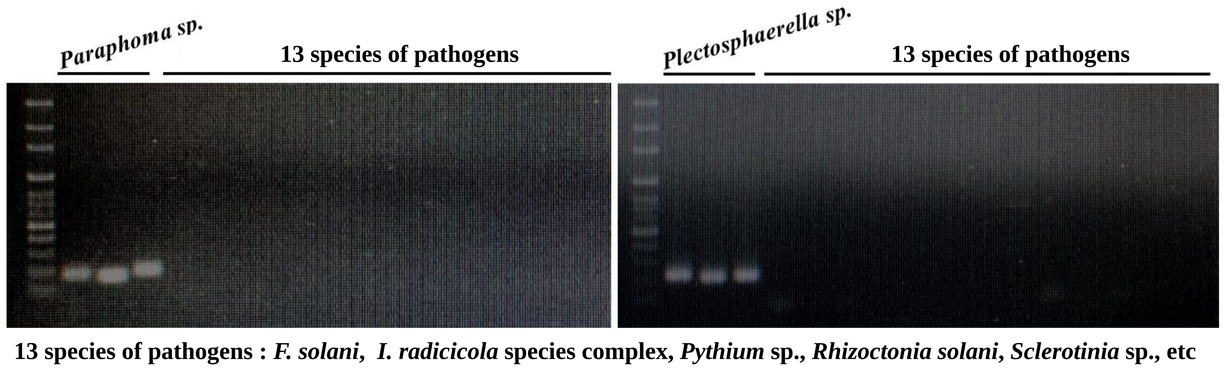
Genome analysis

Genome analysis	*P. radicina*	*P. cucumerina*
Contig No.	8	28
Contig length (bp)	36,109,961	37,426,307
N50 (bp)	6,042,452	1,459,305
GC content (%)	57.44	51.22
Complete BUSCOs (%)	99.1	96.3
Repeat content (%)	2.91	2.97
Functional annotated gene No.	11,198	11,734

Microsatellite

SSR Type	*P. radicina*	*P. cucumerina*
2-mer	921	1335
3-mer	1781	3892
4-mer	1192	3321
5-mer	358	1163
Total	5,155	10,858

Design of specific primer



13 species of pathogens : *F. solani*, *I. radicicola* species complex, *Pythium* sp., *Rhizoctonia solani*, *Sclerotinia* sp., etc

Ⅳ. Summary

- **To explore genetics diversity at novel microsatellite loci, we developed 69 new markers after identifying repeat sequences in the genome of *P. radicina* and *P. cucumerina*.**
- **Based on the PCR band patterns by electrophoresis, these novel microsatellite primers could be classified into three distinct groups showing variation.**