Anti-fungal materials Produced by *Streptomyces albogriseus* Isolated in Korean soil

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Abstract

An anti-fungal material producing actinomycete was isolated from domestic soil. This strain was identified as *Streptomyces albogriseus* by 16S rDNA sequence. YEME (yeast extract 4g, malt extract 10g, glucose 4g, D.W 1ℓ, pH 7.0±0.2) medium was used for production of anti-fungal materials. *S. albogriseus* was cultured in a shaking incubator for 2 weeks at 150 rpm and 25±1℃. An anti-fungal material produced by *S. albogriseus* was identified at 340nm by uv/vis- spectrometer and it showed powerful anti-fungal activity. This is the first report that secondary metabolite produced by *S. albogriseus* showed an activity against phytopathogenic fungi such as *Collectrichum coccodes, Botrytis cinerea, Cladosporium cucumerinum, Didymella bryoniae.*

Introduction

An excessive use of agricultural chemicals for mass production of the crops has resulted in a disturbance of ecological system by polluting soils, streams and groundwater. The residual agricultural chemicals also have a substantial effect on human health (Lee *et al*, 1995). A biological prevention, as a reduction of, has been actively studied (Siegel *et al*, 1977). *Bacillus thuringiensis* has best known as biopesticide . It utilizes antibiotic substances or toxins produced by soil microorganisms (Lachhab, 2001). *Acinetobacter, Serratia, Pseudomonas, Aeromonas, Lactobacillus, Streptomyces* and *Promicromonospora* have also reported as producers of antifungal materials (Sin *et al*, 1995; 1999; Han *et al*, 1999; Paola *et al*, 2000). In this study, actinomycete was isolated from domestic soil and its antifungal effect against crop disease fungi was investigated.

Materials and Methods

1. Isolation of actinomycete

Soil sample was heated at 100℃ for 60 min. and 10 fold diluted. The diluted solution was cultured on Bennett's agar and Bacto-Actinomycete Isolation agar (AIA) at 25±1℃ for 7days (Goodfellow *et al*, 1989).

2. Strain identification by 16S rDNA sequencing

27F (5'-AGAGTTTGATCATGGCTCAG-3') and 1492R(5'-GGATACCTTGTTACGACTT-3') primer were used for 16S rRNA sequencing. PCR condition was denaturation, 94℃, 60sec; annealing, 60℃, 60sec; polymerization, 72℃, 90 sec. The 16S ribosomal DNA of 1400 bp was isolated and purified by 0.8% agarose gel electrophoresis. ABI PRISM 3700 DNA Analyzer

conducted analysis of base sequencing. And then it was compared with ribosomal RNA sequencing of GENEBANK and RDP (RNA database project).

3. Antifungal activity test of selected strain

To confirm the antifungal activity of selected strain against phytopathogenic fungi such as *Collectrichum coccodes* KACC40227, *Botrytis cinerea* KACC40573, *Cladosporium cucumerinum* KACC40576, *Didymella bryoniae* KACC40900, isolated strain was subcultured on YEME (yeast extract 4 g, malt extract 10 g, glucose 4 g, D.W 1ℓ, pH 7.0±0.2), incubated at 25 ± 1℃ for 2 weeks. And secondary metabolite was extracted by ethyl acetate. The antifungal activity was examined by paper disc method.

Results and Discussion

1. Strain identification

The 16S rDNA sequence of isolated strain is shown in fig.1. It was compared with ribosomal RNA sequencing of GENEBANK and RDP (RNA database project). The isolated strain was identified as *Streptomyces albogriseus.*

Scanning electron micrograph of isolated *S. albogriseus* is shown in fig.2.

CTACCATGCAAGTCGAACGATGAACCTCCTTCGGGAGGGGATTAGTGGCGAACGGGTGAG
TAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACC
GGATATGACACGGGGTCGCATGATCTCCGTGTGGAAAGCTCCGGCGGTGAAGGATGAGCC
CGCGGCCTATCAGCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGC
CTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGA
CGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAGAGTGACGGTACCTGCAG
AAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGT
CCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGATGTGAAAGCCCG
GGGCTTAACCCCGGGTCTGCATTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGG
AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGG
ATCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTGGGCGACATTCCACGTCGTCC
GTGCCGCAGCTAACGCATTAAGTTCCC

Fig. 1. The 16S rDNA sequence of isolated *S. albogriseus.*

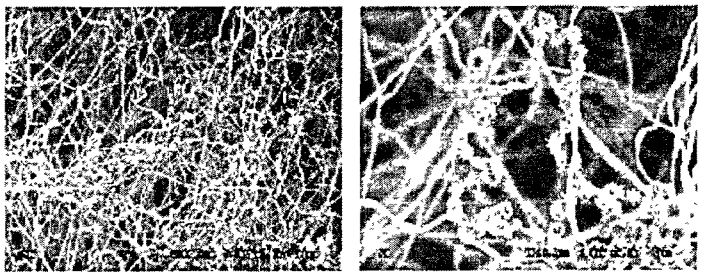

Fig. 2. Scanning electron micrograph of isolated *S. albogriseus.*

2. Extraction of antifungal material

Antigungal material extracted with ethyl acetate formats yellowish colored precipitation. It was identified at 340nm by uv/vis- spectrometer (Spectronic Genesys^{TM} 2PC).

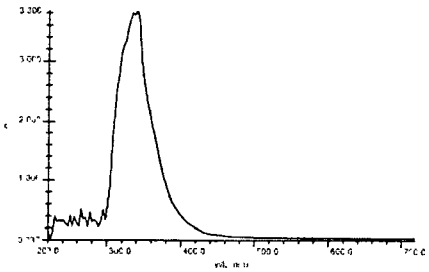

Fig. 3. UV-Spectrum of anti-fungal materials extracted with ethyl acetate.

3. Antifungal activity test

The *C. coccodes* causes black dot root rot and anthracnose, and *B. cinerea* causes gray mold. *C. cucumerinum* and *D. bryoniae* are the source of scab and black rot, respectively.

Fig. 4. shown the antifungal activity of secondary metabolite produced by isolated *S. albogriseus* against phytopathogenic fungi such as *C. coccodes, B. cinerea, C. cucumerinum, D. bryoniae.* Secondary metabolite produced by isolated *S. albogriseus* is believed that it is a pertinent strain to be biopesticide by showing the excellent broad spectrum against phytopathogenic fungi.

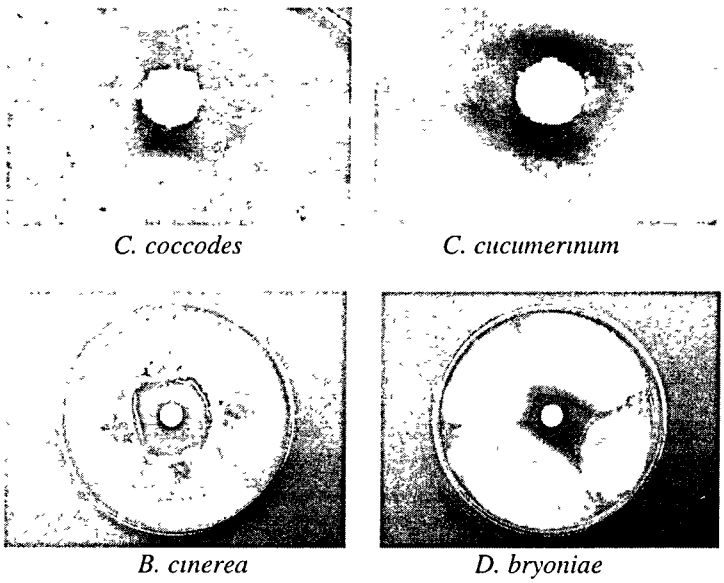

Fig 4. Antifungal activity of crude extract produced by *S. albogriseus*.

Conclusions

1. *S. albogriseus* produced antifungal material was isolated from Korea soil.
2 Antifungal material extracted with ethyl acetate has absorption at 340nm by uv/vis-spectrophotometer.
3. Antifungal material produced by *S. albogriseus* has antifungal activity against phytopathogenic fungi such as *C. coccodes, B. cinerea, C. cucumerinum* and *D. bryoniae.*

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