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**Note**

# An Antifungal Activity of *Streptomyces* sp. against *Cryphonectria parasitica*

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**Strains showing antifungal activity against *Cryphonectria parasitica* were isolated from coast soil of Taean , Korea. Of 152 strains isolated, 6 strains showed antifungal activity *in vitro* against *C. parasitica*. Ta24 strain showed highest activity with 1.6 cm clean inhibition zone. For strain identification, the morphological characteristic and 16S rDNA sequences were determined. Ta24 strain showed 99% homology with *Streptomyces sampsonii* and was identified as *Streptomyces* sp.**

**Key words:** *Streptomyces sp., chestnut blight disease, Cryphonectria parasitica, 16S rDNA, antifungal activity*

Chestnut blight disease caused by *Cryphonectria parasitica* first occurred in New York Zoological Garden in 1904 destroying the chestnut forestry field of East America. Since then , the pathogen has spread to Europe and caused serious damage to the chestnut trees. In Korea, the disease was first reported as chestnut stem blight in 1925.[5,9]

Chestnut trees distributed worldwide are economically important trees, especially in Korea, where the fruits, which contain many nutrients for human health, are exported to Japan. Many chestnut trees were planted from the end of the 1960's to the middle of the 1970's in Korea, approximately about 79,000 ha, yielding 10,000 tons per year. Losses in yields caused by Chestnut blight fungus, *C. parasitica*, has been increasing due to the lack of proper strategy of disease management.

Early attempts at biological control in North America involved the isolation of fungal strains with severely reduced virulence and weakened mycelia growth and sporulation, because they perturb the developmental processes of host.[2-4,6-7] However, hypovirulent *C. parasitica* field isolates exhibit a wide range of variability in the virulence levels. Recently, results of genetic diversity on the hypovirus isolates that hypovirulent *C. parasitica* was useful and stable as biological control agent at a geographic scale were questoned.[8] In Korea, hypovirulent *C. parasitica* also was proposed as one of attempts to protect chestnut trees from chestnut blight fungus. But *C. parasitica* isolated in Korea showed a wide genetic variation that could reduce the effectiveness of biological control. Therefore, in the study attempts were made to isolate bacteria showing antifungal activity for biological control against *C. parasitica*.

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## Materials and Methods

**Bacteria isolation and Morphology.** To isolate strains from soils, serially diluted soil samples were plated on potato dextrose agar plates at 25°C for 3 days, and the resulting colonies were incubated with *C. parasitica* for determination of antifungal activity. Morphological characteristics were observed by FE-SEM (S-4300, Hitachi, Japan) following the procedure of Williams.[11] The strains for SEM observation were cultured on a Petri dish containing Bennet's medium at 28°C for 7 days. Samples were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) at room temperature for 4 h, followed by washing three times at 4°C for 10 min in 0.05 M sodium cacodylate buffer (pH 7.2). Samples were post-fixed with 1% aqueous

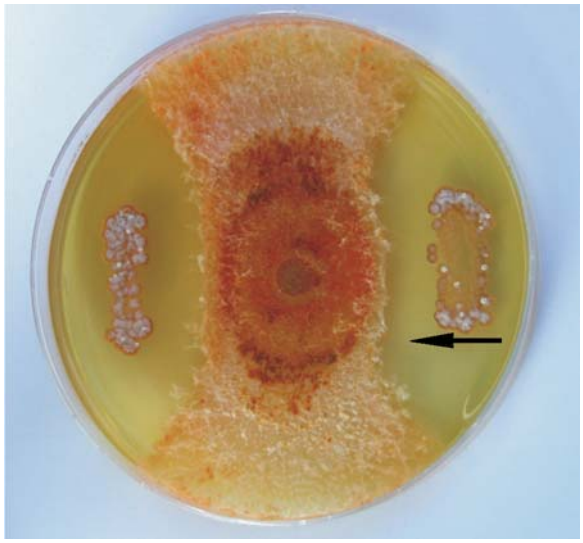

**Fig. 1. Effect of Ta24 isolate on growth inhibition in dual culture. Clear zone showing growth inhibition of mycelium is indicated by arrow.**

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A24  AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60
St   AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAAC 60

A24  GATGAACCGCTTTCGGGCGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTG 120
st   GATGAACCGCTTTCGGGCGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTG 120

A24  CCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATATGACTGTCCATCG 180
st   CCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATATGACTGTCCATCG 180

A24  CATGGTGGATGGTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTT 240
st   CATGGTGGATGGTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTTGTT 240

A24  GGTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCA 300
st   GGTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCA 300

A24  CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA 360
st   CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA 360

A24  ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAAC 420
st   ATGGGCGAAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAAC 420

A24  CTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTA 480
st   CTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTA 480

A24  CGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAA 540
st   CGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAA 540

A24  AGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTG 600
st   AGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTG 600

A24  CAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTG 660
st   CAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTG 660

A24  AAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGA 720
st   AAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTGA 720

A24  CGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGT 780
st   CGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGT 780

A24  AAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATT 840
st   AAACGGTGGGCACTAGGTGTGGGCAACATTCCACGTTGTCCGTGCCGCAGCTAACGCATT 840

A24  AAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCC 900
st   AAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCC 900

A24  CGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCT 960
st   CGCACAAGCGGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCT 960

A24  TGACATACACCGGAAACGCCTGGAGACAGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTG 1020
st   TGACATACACCGGAAACGTCTGGAGACAGGCGCCCCCTTGTGGTCGGTGTACAGGTGGTG 1020

A24  CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC 1080
st   CATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC 1080

A24  CTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGG 1140
st   CTTGTCCCGTGTTGCCAGCAGGCCCTTGTGGTGCTGGGGACTCACGGGAGACCGCCGGGG 1140

A24  TCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGC 1200
st   TCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGC 1200

A24  ACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAA 1260
st   ACACGTGCTACAATGGCCGGTACAATGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAA 1260

A24  AAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTA 1320
st   AAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTA 1320

A24  GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG 1380
st   GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG 1380

A24  TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGC 1440
st   TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGC 1440

A24  TGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTAATCACTAGT 1500
st   TGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGT 1500

A24  GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG 1380
st   GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG 1380

A24  TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGC 1440
st   TCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGC 1440

A24  TGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTAATCACTAGT 1500
st   TGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGT 1500

A24  GAATTCGCGGCCGCCTGCAGGTC 1523
st   GCGGCTG-GATCACCTCCTTTCT 1522
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**Fig. 2. BLAST search results (A24 isolated strain; St: *Streptomyces sampsonii*).**

osmium tetraoxide in 0.05 M sodium cacodylate buffer (pH 7.2 at 4°C) for 2 h, following two times washing with distilled water. Samples were serially dehydrated in ethanol (30, 50, 70, 80, 90, 100, 100, 100%), treated with isoamyl acetate for 15 min two times, critical point-dried in liquid $CO_2$ using the Balzers CPD 010 (Balzers Instruments, Liechtenstein), mounted on aluminum stubs, and sputter-coated with gold and palladium using the Polaron SEM Coating Unit E5100 (Thermo VG Scientific, Beverly, MA, USA).

**16S rDNA sequence analysis.** To identify strains showing antifungal activity, genomic DNA was isolated using the genomic DNA extraction kit (QIAGEN, Hilden, Germany) for 16S rDNA analysis. 16S rDNA was amplified by PCR using a PCR reaction kit (Bioneer, Daejon, Korea). The primer sequences were: forward, 5'-AGA GTT TGA TCC TGG CTC AG-3'; and reverse, 5'-ACG GCT ACC TTG TTA CGA CTT-3'. Thermal cycle conditions were as follows: 1 cycle of denaturation at 94°C for 5 min; followed by 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min; and final extension at 72°C for 10 min. The PCR products were cloned using a pGEM-T cloning kit (Promega, Madison, WI, USA) according to the manufacture's instruction. The cloned fragment was directly sequenced using a Taq DyeDeoxy terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and analyzed with an ABI PRISM 3700 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The internal sequences of the PCR products were determined using four internal primers: p510r 5'-TAT TAC CGC GGC

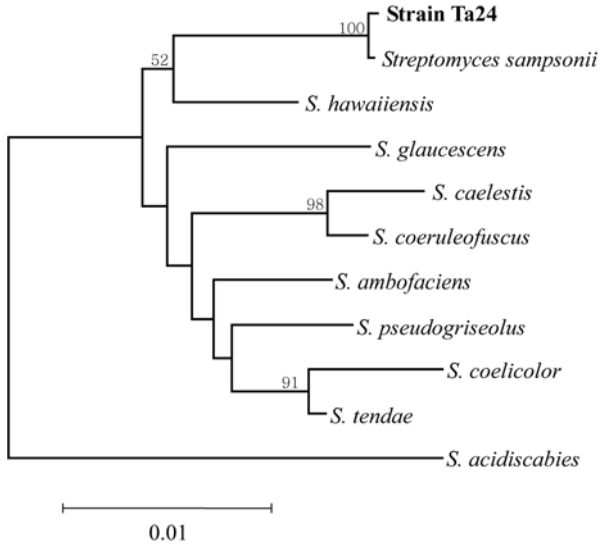


**Fig. 3. Phylogenetic position of strain Ta24 within the genus *Streptomyces*. The branching pattern was generated by the neighbor-joining method. The value at the inter nodes indicates the level of bootstrao support with 1000 replication.**

TGC TG-3', p364f 5'-GGC AGC AGT GGG GAA TAT TG-3', p783f 5'-TAG ATA CCC TGG TAG TCC AC-3', and p1037f 5'-TCG TCA GCT CGT GTC GTG AG-3'. The determined sequences were analyzed by the homology search of the BLAST program at National Center for Biotechnology Information and aligned with ClustalW software.

## Results and Discussion

Strains showing antifungal activities against *C. parasitica* were isolated from soil of Taean, Korea. Of 152 strains isolated, 6 showed antifungal activities *in vitro* against *C. parasitica*. The strains were cultured with *C. parasitica* for growth inhibition assay by dual culture. The results of growth inhibition indicated that the diameters of the clean zones of strains Ta1, Ta3, Ta4, Ta9, Ta16, and Ta24 were 1, 1.5, 1.4, 0.8, 1.3, and 1.6 cm, respectively. Strain Ta 24 showed the highest growth inhibition against the chestnut tree pathogen (Fig. 1). For the identification of the strain, the morphological characteristic and 16S rDNA sequences were determined. The 16S rDNA sequences of strain Ta24 showed 99% homology with *Streptomyces sampsonii* by the BLAST program (Fig. 2) and strain was thus identified as *Streptomyces* sp. The phylogenetic relationship based on the 16S rDNA sequences was created by the neighor-joining method (Fig. 3).[10] The morphological characterization was obtained by a previous reported method (Fig. 4).

*Streptomycetes* are widely used in industries due to their ability to produce chemical compound, antibiotics, and antitumor agent.[1] In particular, *S. sampsonii* could protect the plants from the disease caused by fungal genus.[12] Therefore, strain Ta24 as biological control could be used to protect chestnut trees from *C. parasitica*.

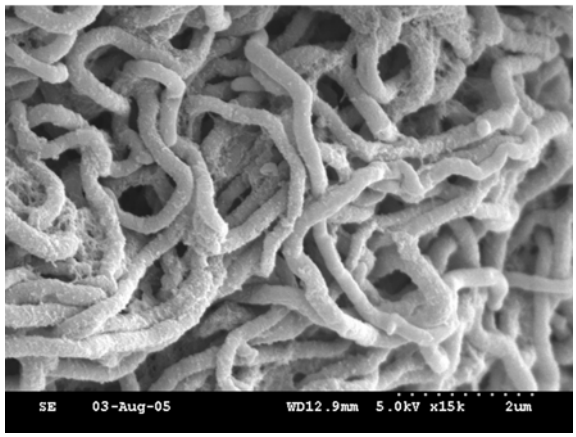


**Fig. 4. Scanning electron micrographs showing typical morphology of the genus *Streptomyces*.**

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