# ISOLATION AND CHARACTERISATION OF ENDOPHYTIC STREPTOMYCETES FROM RICE ROOTS AS A ROADMAP TO NOVEL BIOACTIVE COMPOUNDS

Byung-Yong Kim*[1], Mun-Hyung Bae[2], Jae-Hyung Ahn[1], Hang-Yeon Weon[1], Dong-Chan Oh[2] and Jaekyeong Song[1],

[1]Division of Agricultural Microbiology, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea (Correspondence: greg6044@gmail.com)
[2]National Products Research Institute, College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea

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## Introduction

Most clinically and agricultural relevant antibiotics originate from microorganisms, notably streptomycetes. New approach is required to investigate novel sources which have not been explored for their potential natural products. Microbial symbioses are widespread and largely unexplored for natural products.

Endophytic bacteria, which exist in the inner tissues of living plants, have attracted increasing attention among microbiologists and chemists due to enormous diversity of species and compounds with different functions.

*Objectives*

- Exploration of endophytic streptomycetes diversity in rice roots.
- Searching for promising new bioactive compounds from unexplored habitats, rice roots for sustainable agriculture.

## Methods

- Sampling site: The experimental rice field of NAAS, RDA in Suwon, Korea.
- Selective Isolation: Starch casein agar supplemented with nystatin and cycloheximide (25 μg/ml each).
- DNA extraction using bead-beating method.
- Cultivation: GYM media at 28 °C for 1 week.
-16S rRNA gene analysis & PKS & NRPS gene screening
- Dendrogram: Eztaxon server & MEGA (ver 4.0).
- LC-MS analysis for metabolites screening.

## Results

- All Isolates were tested for antibiotic production against different Gram-negative and positive bacteria and some plant pathogenic fungi.
- It was found that all isolates inhibited three or more plant-pathogenic fungi including *Botrytis cinerea*, *Colletotrichum gloeosporioides, Pyricularia grisea* and *Sclerotinia sclerotiorum*. They also inhibited Gram-positive bacteria such as *B. subtilis* and *S. aureus*.
- Moreover, they showed positive reaction for PCR detection targeting biosynthetic gene clusters, namely NRPS and PKS genes.
- After screening of secondary metabolites from these isolates with LC-MS, the compounds were supposed to be the similar compounds with ansamycin antibiotics, ansatrienin and setomimycin.

Table1. Antimicrobial properties of endophytic *Streptomyces* stains isolated from rice root.

| Strains | *S. sclerotiorum* | *C. gloeosporioides* | *D. bryoniae* | *B. cinerea* | *F. oxysporum* | *P. grisea* | *P. ultimum* | *B. oryzae* | *F. solani* | *R. solani* | *B. subtilis* | *E. coli* | *S. aureus* | *P. carotovorum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BYK1006 | | +++ | | ++ | | +++ | | | | | | | + | |
| BYK1007 | +++ | +++ | ++ | +++ | | +++ | ++ | | | ++ | ++ | ++ | ++ | ++ |
| BYK1008 | ++ | +++ | | ++ | | ++ | | | ++ | | | | ++ | |
| BYK1009 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | | | + | | | |

Table2. PCR detection of polyketide synthase and non-ribosomal peptide synthetases genes in endophytic isolates.

| | Target genes | | | |
|---|---|---|---|---|
| | PKS-I | NRPS | degKS | PKS-II |
| BYK1006 | + | + | - | |
| BYK1007 | + | + | + | + |
| BYK1008 | + | + | | + |
| BYK1009 | + | + | + | + |

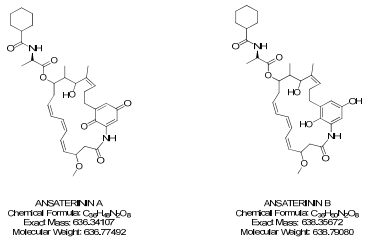


Fig.1. Putatively identified medtabolites of endophytic *Streptomyces* strains, BYK1006 and BYK 1007.

## Conclusions

All isolates, identified to the genus *Streptomyces* based on the genetic and morphological characters, showed strong antimicrobial properties and were detected for PKS and NRPS genes.

This study suggests that diverse *Streptomyces* strains can be isolated from rice roots, and promising novel bioactive compounds can be discovered from these entophytic habitats.

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