





Genome-wide association study and functional analysis of non-ribosomal peptide synthetases in biocontrol fungus *Clonostachys rosea*

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Non-ribosomal peptides (NRPs)

- Class of peptide secondary metabolites
- NRPs are synthesized by NRP synthetases (NRPSs)







ECFG15

adenylation domain

peptidyl carrier protein

condensation domain

Finking and Marahiel (2004) Annu Rev Microbiol.

Clonostachys rosea (Hypocreales)

• *Clonostachys rosea* is a necrotrophic mycoparasite with broad host range



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Zhang et al. (2008) Appl Microbiol Biotechnol.

Collection of *C. rosea* **strains**





Total 120 C. rosea strains collected, WGS performed for 53 strains

Broberg et al. (2018) Front Microbiol.

Hypotheses



• There is variation in the ability to produce nematicidal compounds between different *C. rosea* strains under *in vitro* conditions

• The *in vitro* antagonistic ability is correlated with biological control of plant parasitic nematodes

Antibiosis assay



• 279 SNPs were associated (Ifsr <10⁻¹⁰) with *in vitro* antagonism of *Pratylenchus penetrans*

• Nps4 and nps5 selected for detailed study





Iqbal et al. (submitted)

80

70

Biocontrol efficacy against nematodes



High *in vitro* antagonism strains

Low in vitro antagonism strains (

Control

ECFG15

In vitro antagonism vs biocontrol



bc

1882 ontrol



High *in vitro* antagonism strains Low *in vitro* antagonism strains

Iqbal et al. (submitted)



Control

GWAS analysis identified two NRPS



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Growth and conidiation rate



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156 to 182% higher conidiation rate observed in $\Delta nps4$ and $\Delta nps5$ strains compared with WT



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observed in $\Delta nps4$ and $\Delta nps5$ strains compared with WT

Iqbal et al. (*submitted*)

3.5

3

Δnps reduced antagonism on nematodes





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24-well plastic plate assay

23% reduction observed in mortality of nematodes compared with WT

Reduced biocontrol efficacy on nematodes ECFG15



20% increase observed in recovered nematodes compared with WT

Plant-parasitic nematodes

- Heterodera (J2)
- Pratylenchus
- Merlinius
- Boleodorus
- Rotylenchus
- Helicotylenchus
- Tylenchorhnchus
- Paratylenchus

Non plant-parasitic nematodes

- Bacterivorous
- Fungivorous
- Omnivorous

Iqbal et al. (submitted)

Reduced biocontrol efficacy on nematodes ECFG15 ROME · ITALY 2020



29% increase observed in recovered nematodes compared with WT

Iqbal et al. (submitted)

Summary



- Intrinsic differences exists among *C. rosea* strains in terms of antagonism against *P. penetrans*
- A weak correlation was found between *in vitro* antagonism and biocontrol of nematodes (soil)
- *Nps4* and *nps5* gene products influence:
 - Growth and conidiation
 - *In vitro* antagonism against nematodes
 - Biocontrol of nematode root diseases on wheat

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