Soil bacteria with biocontrol potential for ginseng replant disease

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INTRODUCTION

American ginseng (*Panax quinquefolius* L.) production is limited by its inability to grow in the same field due to lingering deleterious effects when ginseng was previously grown, known as ginseng replant disease (GRD) (Westerveld & Shi, 2021). This disease is most often associated with the root rot pathogen *Ilyonectria mors-panacis* (A.A.Hilderbr.) A. Cabral & Crous, formerly known as *Cylindrocarpon destructans* (Zinssm.) Scholten.

One approach to controlling this pathogen would be to use rhizobacteria isolated from a ginseng field as biocontrol agents that could directly inhibit the pathogen and increase ginseng survival. For example, a biocontrol agent for apple replant disease, Enterobacter aerogenes B8, also significantly reduced ginseng seedling mortality when applied to replant soil (Li, 1993). Many biocontrol rhizobacteria can also act as plant growth promoters (PGPRs).

To assess traits of interest, rhizobacteria genomes can be sequenced and investigated for the presence and copy number of genes that may be responsible for these.

METHODS

Soil bacteria were isolated and identified from soil samples from a ginseng field at 73 days post-harvest (dph) via serial dilution in trypticase-soy-agar (TSA) media and 16S rRNA sequencing, respectively. To test for potential biocontrol activity, bacterial isolates were bioconfronted with different fungal isolates of *Ilyonectria* sp., pathogenic to ginseng.

A volume of 20 µl of bacterial cells at $1x10^9$ $cell.ml^{-1}$ was placed in the center of potato-dextrose-agar (PDA) plates and four 1 cm^2 plugs of 4 week-old mycelia of each *I. robusta* (NR4.BC16-1), *I. mors-panacis* (ND3.P14-1) and *I. mors-panacis* (ND4.Z15) were placed equally spaced at 2.5 cm away from the bacteria, and plates were incubated at room temperature in the dark. The area of mycelial growth was measured at 3 weeks via ImageJ software (Schneider et al., 2012) and compared via t-test (p = 0.05).

The genome of the bacterial isolates were sequenced via Illumina NovaSeq technology, gene annotation was done via RAST server and the results were analyzed for PGPR traits, secondary metabolites production and lytic enzymes via literature search, antiSMASH and CAZymes databases, respectively.

RESULTS

A total of 23 bacterial strains were isolated from ginseng soil with different phenotypes, belonging to 15 different species, being *Bacillus* and *Pseudomonas* the most common genera.

Bioconfrontation culture assays of *Bacillus subtilis* LWO2.73, *Bacillus megaterium* LWO4.73, *Pseudomonas putida* MWO1.73 and *Pantoea agglomerans* LYT1.73 showed that ND3.P14-1 was significantly inhibited only by LWO2.73, NR1.BC16-1 was significantly inhibited only by LWO4.73, and no isolate significantly inhibited ND4.Z15. Genes related to PRPG traits and biocontrol activities were found in several genomes with varying copy numbers.

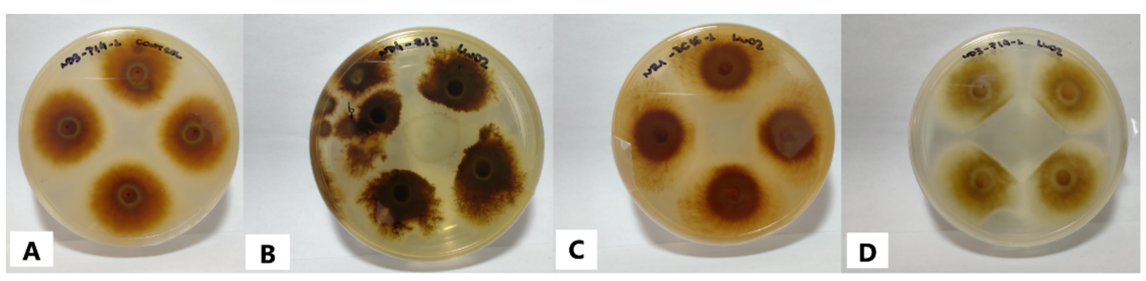

Figure 1. Bioconfrontation of soil bacteria against *Ilyonectria* isolates. A: Control plate for ND3.P14-1; B: LWO2.73 against ND4.Z15 (not significant); C: LWO2.73 against NR1.BC16-1 (not significant); D: LWO2.73 against ND3.P14 (significant at p=0.05).

RESULTS

Table 1. Gene annotations of four bacterial strains from ginseng soil. Each value in the table represents the number of genes detected for each category.

		MWO2.10	MWO2.73	MWO2.231	MW12.73
CAZyme	Glycoside Hydrolases	101	130	162	74
	Polysaccharide Lyases	14	15	17	2
antiSMASH	Secondary metabolites	Keywimsyn, carotenoid, entothonella, fellutamide B, bacillibactin	Keywimsyn, carotenoid, entothonella, fellutamide B, bacillibactin	Keywimsyn, carotenoid, entothonella, fellutamide B, bacillibactin	Paenidonin, carotenoid, fosfomycin, alkylresorcinol, amylocyclicin, micrococcin P1, microcin C7, eremophilene, petrobactin, bacilysin
RAST server	Phosphate solubilization and transport	5	5	5	3
	IAA synthesis	1	1	1	1
	Auxin synthesis	0	0	0	0
	Siderophore biosynthesis (BCGs), protein monoxygenase	0	0	0	0
	Pyoverdine synthase PvdF, biosynthesis of pyoverdine siderophores	4	5	5	2
	ACC deaminase activity	1	1	1	1
	Acetoin and butanediol synthesis (2,3,butanediol dehydrogenase (acetoin, butanediol metabolism))	0	0	0	0
	Chitinase production	3	3	3	0
	4-hydroxybenzoate production	0	0	0	0
	Pyocin	0	0	0	0
	H2S production	0	0	0	0
	Quorum sensing	0	0	0	2
	Heat shock proteins	4	4	4	5
	Cold shock proteins	3	3	3	18
	Glycine-betaine production	0	0	0	11
	Peroxidases	4	4	4	4
	Catalases	9	9	9	4
	Superoxide dismutase (SOD)	1	1	1	1
	GABA production	2	2	2	7
	Pyrroloquinoline quinone cofactor and coenzyme PQQ synthesis proteins	1	1	1	0
	Phenazine antibiotic biosynthesis	1	1	1	2
	Trehalose metabolism	3	3	3	5
	Heavy metal sensor histidine kinase (cobalt, zinc, cadmium resistance)	1	1	1	1
	Nitrogen metabolism and transport	0	0	0	11
	Sulfur metabolism and transport	0	0	0	1
	Zinc solubilization and transport	0	0	0	1
	Glucose dehydrogenase	0	0	0	1
	D-cysteine desulfhydrase	0	0	0	0

FUTURE ACTIONS

Genomes and bioconfrontation assays will be determined for the entire collection of isolates from 73 dph soil. The most promising isolates will be added to replant soil and ginseng seeds will be then planted to determinate which isolates increase ginseng seed germination and seedling survival.

REFERENCES

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