In vitro Screening for Biocontrol Potential Abilities of Ohio Bacterial Isolates over Tomato Pathogens

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Abstract

In vitro inhibition of pathogen growth by other microorganisms can be used to initially select isolates with biocontrol potential. We set out to identify spore-forming bacteria with the potential to control diverse tomato pathogens. Using a novel isolation strategy, 1500 spore-forming isolates were obtained from corn and soybeans grown in different Ohio soils. From this collection 167 isolates were selected based on their ability to inhibit the growth of *Fusarium spp*. Phylogenetic analysis of 16S rDNA sequences revealed that 51% of the isolates belong to one species (A), 42% were another species (B) and the remaining 8% belong to other two species (C or D). Haplotype analyses were used to select 23 distinct genotypes. Based on inhibition over *Botrytis cinerea* and their genotype, 8 different isolates were selected for further studies. These were screened for inhibition activity against six other tomato pathogens (*Alternaria solani, Colletotrichum coccodes, Fusarium oxysporum, Phytophthora capsici, Xanthomonas campestris and Pseudomonas syringae*). Isolates A1, A2, A3, B2, and D1 each inhibited the growth of three or more pathogens ($P<0.05$). Additionally, fermentation products of strains A2, B2, and D1 inhibited *X. campestris* growth ($P<0.1$); while those of strains A2 and B2 inhibited *P. syringae* ($P<0.1$). Similarly, extracts of strains A2, A3, B2 and D1 inhibited Fusarium oxysporum growth ($P<0.05$) and only extracts of strain A3 inhibited *P. capsici* ($P<0.05$). Thus, from a collection of 1500 bacteria we selected 5 candidate strains with broad-spectrum antimicrobial activities for further testing and formulation development.

Introduction

❖Spore-forming bacteria are promising candidates for developing biopesticides because of their resistance to heat and desiccation (Gnanamanickam, 2002). *In vitro* inhibition bioassays (fig 1) can be used to identify isolates with potential to control pathogen establishment. Moreover, this process is an approach in which a wide spectrum of isolates can be tested prior to test *in planta;* by this means the number of isolates to be tested *in planta* is narrowed and therefore labor and costs are minimized. This process in combination with genotypic characterization of the isolates obtained can be used to avoid further screening of redundant isolates, assure that diverse groups are tested and determine subspecies variation. Eventually, the isolates with the greatest ability to control tomato disease development could be formulated as a commercial product that can be provided to Ohio growers.

Objective

❖ Identify spore-forming bacteria native to Ohio with biocontrol potential over a wide collection of tomato pathogens by an *in vitro* inhibition activity test.

Fig. 1. Example of *in vitro* inhibition of *Fusarium oxysporum* by several bacterial isolates.
1. Negative control (water)
2. No Inhibition of fungal growth by the bacteria.
3. Fungal growth inhibited by bacteria

Methodology

Isolation of spore forming bacterial isolates from rhizosphere soil.

↓

Test *in vitro* inhibition over *F. oxysporum* (scale 0-2; 0 no inhibition, 2 >1mm of inhibition)

↓

Sequence (16s rDNA) 167 isolates with inhibition capacity.

↓

Screen inhibition abilities of 23 isolates over *B. cinerea* in 1/5X potato dextrose agar media (PDA)

↓

Screen 8 isolates on their inhibition abilities over 6 pathogens: *A. solani, C. coccodes, F. oxysporum, P. capsici* in 1/5PDA and *X. campestris* and *P. syringae in* 1/10X tryptic soy agar media.

↓

Test inhibition abilities of the fermentation extracts of the 5 top isolates over 4 pathogens (*F. oxysporum, P. capsici, X. campestris* and *P. syringae*).

Results

❖**1500 spore-forming isolates** from Ohio soils grown with corn and soybeans were obtained.
❖**167 isolates** with the highest inhibition rate over growth of *Fusarium* spp. were selected.
❖**23 distinct genotypes** that belong to 4 species (A,B,C,D)[z] were identified based on haplotype difference of the 16S rDNA sequence (Table1).

Table 1. Diversity and distribution of spore-forming bacilli recovered from 10 Ohio soils.

Species[1]	Bacterial Isolates belonging to each species (%)	Soils from which each species was isolated (%)	Different haplotypes identified (n°)[2]	No.Soils/Haplotype[3]	No.Haplotypes/ Soil[4]
A	51	90	6	7,7,1,1,1,1	1.9
B	42	70	5	7,2,2,1,1	1.3
C	4	20	1	2	1.0
D	4	10	1	1	1.0

[1]The taxonomical classification (i.e genus and species) of the isolates is not provided because they are patent pending.

[2]Total number of different haplotypes identified within each species.

[3]Incidence of haplotypes in soils within each species.

[4]Proportion of the total number of haplotypes isolated from each soil.

❖**8 isolates** were selected based on their genotypic diversity and their inhibition potential over *B. cinerea:* A1,A2,A3, B1,B2,B3,C1,and D1
❖**5 isolates** were selected based on their genotypic diversity and their inhibition potential over *A. solani, C. coccodes, F. oxysporum, P. capsici, X. campestris* and *P. syringae* (table 2): A1,A2,A3,B2 and D1.

Table 2. Inhibition of tomato pathogens by spore-forming bacterial isolates; the ** represents the isolates that had greater inhibition than the untreated negative control according to the Mann-Whitney test at a $P \leq 0.05$(n=3).[1]

	Pathogen						
Bacterial Isolate	*P. syringae*	*X. campestris*	*P. capsici*	*B. cinerea*	*A. solani*	*C. coccodes*	*F. oxysporum*
A1	**	**	**	**	**	**	**
A2	**	**	**	**			**
A3	**		**	**	**	**	**
B1	**	**					
B2	**	**			**		
B3	**						
C1	**						
D1		**	**	**			**

[1] Inhibition scale from 2-0; 2 clearing zone between pathogen and bacterial experimental (>1mm), 1 intermediate inhibition(<1mm), 0 no inhibition

❖ **Fermentation extracts** inhibition (fig 2)
-A2,A3,B2 and D1 inhibited *F. oxysporum*
-A3 inhibited *P. capsici*
-A2,B2, and D1 inhibit *X. campestris*
-A2 and B2 inhibited *P. syringae* (fig.2)

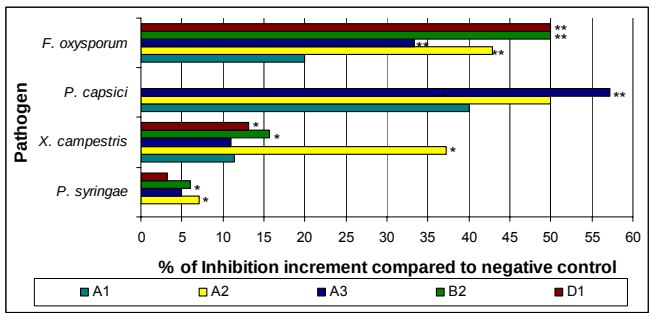

Fig. 2 Inhibition of tomato pathogens by bacterial fermentation extracts showed in percent of increment compared to the negative control (n=3).[1]

[1] Median values of the treated samples assayed within a pathogen differ significantly from the negative control according to the Mann-Whitney confidence interval test; * $P \leq 0.1$, ** $P \leq 0.05$. Data not shown for values in which inhibition was not observed.

Conclusions

❖From an initial collection of 1500, we identified 5 spore-forming bacterial isolates native to the state of Ohio with biocontrol potential over a broad spectrum set of tomato pathogens.

❖Inhibition of the pathogens was either sustained or variable with the fermentation products; which suggested inhibition variability due to environmental conditions.

❖A difference in inhibition aptitudes was observed at the sub-species level; consequently, strengthening the importance of *in vitro* inhibition screening for the identification of the isolates with the strongest inhibition potential.

❖Future work will concentrate on testing the selected isolates in greenhouse and field conditions.

References

❖Gnanamanickam, S. S. editor. Biological control of crop diseases. New York : Marcel Dekker, Inc., 2002. 446p

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[z] The taxonomical classification (i.e genus and species) of the isolates is not provided because they are patent pending.