Bacterial endophyte isolated from corn kernels inhibits the growth of a fumonisin-producing *Fusarium verticillioides*

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INTRODUCTION

Strains of *Fusarium verticillioides* produce mycotoxins such as fumonisins, which are common contaminants of maize and maize-based products worldwide. Fumonisins are considered to be hazardous for human and animal health, due to their nephrotoxic, hepatotoxic, immunosuppressive and carcinogenic activity. Biological control of *Fusarium* infection is of great interest for food safety and could contribute towards reducing the use of toxic chemicals with fungicide activity.

Many plant-associated bacteria, both epiphytes and endophytes, are widely recognized as agents of biological control against plant diseases and infections (Compant *et al*. 2010). Numerous phylogenetically diverse bacterial strains showing inhibitory activity against different fungi have been isolated (Compant *et al.* 2005, Dalié *et al*. 2010, Yoshida *et al.* 2012).

In this study endophytic bacteria from kernels of two corn inbred lines, one resistant (A509) and the other one sensitive (EP42), to fumonisin-producing *Fusarium* spp. (Butrón *et al*. 2006) were cultivated. The inhibition of *Fusarium* growth by selected bacterial isolates was investigated.

MATERIAL AND METHODS

Corn kernels (lines A509 and EP42)

Soaking: sterile H_2O, 48h

Surface sterilization: 3 min 1% NaClO + drops Tween 40 or 3 min 3% NaClO + drops Tween 40

Washing: sterile H_2O, 3 times

Maceration: 2 min medium speed Polytron PT1200E (Kinematica AG)

Ten-fold serial dilution Plating in EF869D medium

Incubation at 28°C Recultivation of different colony morphologies (1-10 representatives)

Genotyping by BOX-PCR Selection of representative isolates

Table 1. Media used in this study

EF869D	g L^{-1}	SNA	g L^{-1}	284	g L^{-1}
Potato peptone	0.5	KH_2PO_4	1	Tris HCl	6.06
Soya peptone	0.5	KNO_3	1	NaCl	4.68
Yeast extract	0.25	$MgSO_4 \cdot 7H_2O$	0.5	KCl	1.49
NaCl	0.5	KCl	0.5	NH_4Cl	1.07
Glucose	0.1	Glucose	0.2	Na_2SO4	0.43
$CaCl_2 \cdot 2H_2O$	0.034	Sacarose	0.2	$MgCl_2\ 6H_2O$	0.20
pH	6.7-7.0	pH	4.5-4.6	$CaCl_2\ 2H_2O$	0.03
				Na_2HPO_4	0,04
				$Fe^{III}NH_4$citrate, 17% Fe	0,005
				TE solution	1 ml
				C sources	See note
				pH	6.5-6.8

Note:
C sources (per liter): Sodium salts of lactate, 0.35 g; gluconate, 0.62 g and succinate, 0.42 g; glucose, 0.52 g and fructose, 0.54 g. TE: trace elements solution SL6 www.dsmz.de Medium 284AV: 284 supplemented: 10 ml L^{-1} BME aminoacids 50X and 5 mL^{-1} of BME vitamins 100X (Sigma-Aldrich). Cycloheximide was added when necessary (cultivation of endophytes)

Inhibition experiments:

A) Co-cultivation of selected isolates and fumonisin-producing *Fusarium* spp. in plates with EF869D, 284, 284AV or SNA media.

B) Step 1- Cultivation of selected isolates in EF869D, 284, 284AV or SNA liquid media in the presence and absence of *Fusarium.*
Step 2- Growth of *Fusarium* spp. on plates in the presence of up to 300 µl of cell-free culture supernatants.

RESULTS AND DISCUSSION

32 endophytic isolates, showing 7 different BOX-PCR profiles, were obtained from resistant line A509. 16 isolates, grouped in 6 BOX-PCR profiles, were cultivated from line EP42.

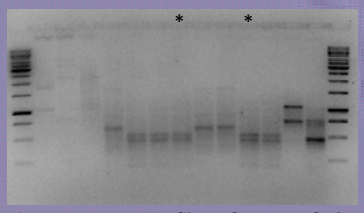

Fig 1. BOX-PCR profiles of some of the endophytic isolates obtained. Lines marked with * correspond to the isolates P3R1 and P3R2 used in this study.

Four isolates of the strain P3R (BOX-PCR profile) from the resistant line A509 strongly inhibited the growth of a fumonisin-producing *Fusarium* spp. on plates with different culture media.

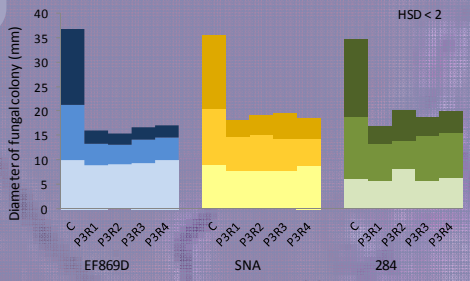

Fig 3. Fungal growth in the absence (C) and presence of 4 different isolates of the strain P3R (P3R1, P3R2, P3R3 and P3R4). Light, medium and high colour intensity correspond to measurements at 24h, 48h and 72h after co-inoculation.
HSD: Honestly significant difference.

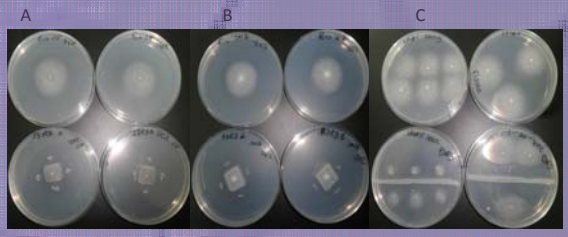

Fig 4. Inhibition of *Fusarium* growth by isolated strain P3R when fungus and bacteria were inoculated simultaneously in EF869D (A) or SNA (B) plates and incubated for 72h and when inoculated at 0.5, 1 and 2 cm (C) from bacteria previously grown in 284AV plates and incubated for 60h. The plates in the first row show the growth of *Fusarium* in the absence of bacteria.

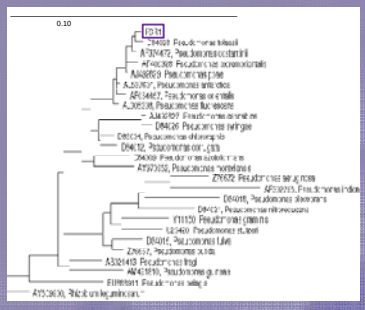

Fig 2. Phylogenetic tree built with 16S rRNA sequences of some *Pseudomonas* type strains and that of isolate P3R1.
Tree was built using Neighbourjoining method and the filter pos_var_ssuref (www.arb-home.de). The 16S rRNA partial sequence (754 bp) from isolate P3R1 was included in the tree by Parsimony method.

P3R isolates produced fluorescent pigments (growth on King B agar) and were identified by partial sequencing of 16S rRNA, as members of the genus *Pseudomonas*. P3R isolates were closely related to *P. tolaasii,*

Fusarium growth was not affected by metabolites present in non-concentrated cell-free supernatant of liquid P3R cultures.

None of the other isolates tested (1 representative of each BOX-PCR group) showed effect on the growth of *Fusarium* spp. on agar plates.

CONCLUSION

The endophytic strain of Pseudomonas P3R may play a role in the resistance of the corn line A509 to *Fusarium* infection and may be useful as biocontrol agent against plant diseases and grain contamination caused by this fungus, although additional and extensive research is needed to confirm this hypothesis.

REFERENCES: Butrón et al. (2006) J. Agr. Food Chem. 54, 6113-6117. Compant et al. (2005) Appl. Environ. Microbiol. 71, 4951-4959. Compant et al. (2010) Soil Biol. Biochem. Dalié et al. (2010) Food Control 21, 370-380. Yoshida et al. (2012) Microb. Ecol. 64, 214-225.
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Background picture from http://phil.cdc.gov/