

# Antagonistic effects of *Bacillus subtilis* bacteria on growth of soil borne plant pathogenic fungi in laboratory tests

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## Introduction

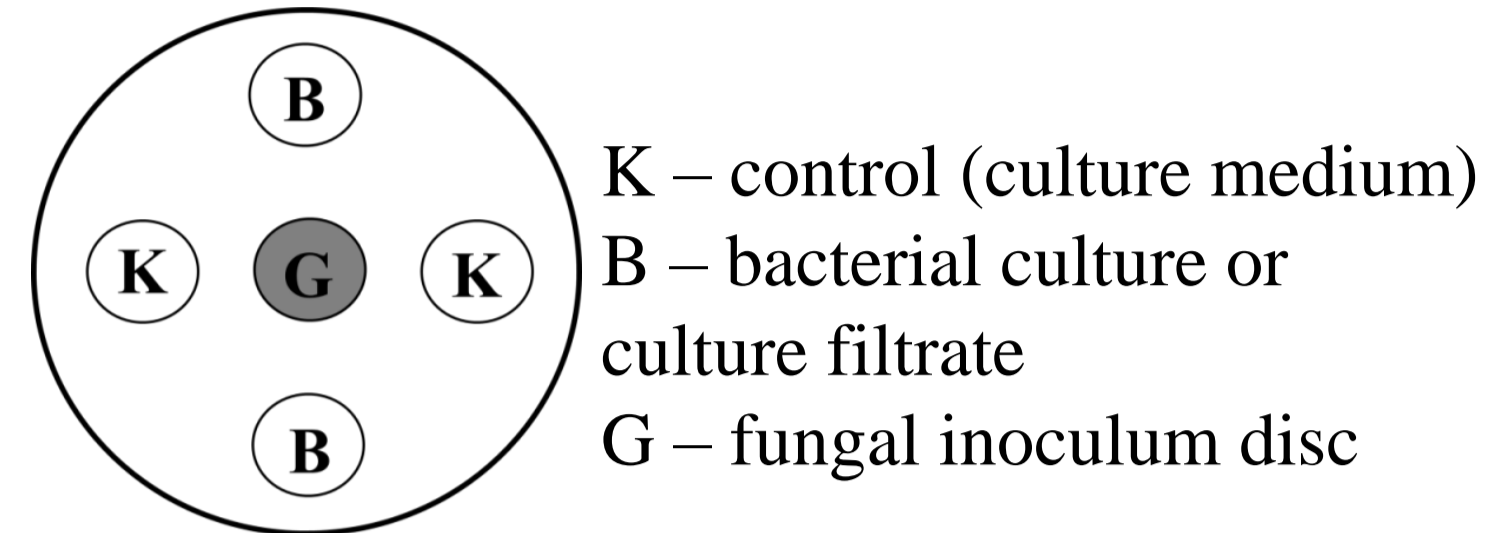
The sustainability of agricultural production and the ever so intensifying demand for healthy and clean foodstuff badly urge the reduction of fungicide usage in field. Environment friendly and at the same time cost effective biological solutions are highly required in field crop production to replace chemical fungicides. Microbial soil inoculants – composed of bacteria that are able to inhibit important soil borne plant pathogenic fungi, like *Fusarium*, *Sclerotinia*, *Alternaria* – represent useful tool to diminish the fungal damage in field.

Our aim was to study the inhibitory effect of *Bacillus subtilis* soil bacterium strain on *in vitro* growth of *Fusarium graminearum*, *Sclerotinia sclerotiorum* and *Alternaria alternata* isolates, in laboratory tests. Both bacterial strain and fungal isolates were obtained from Hungarian soils.



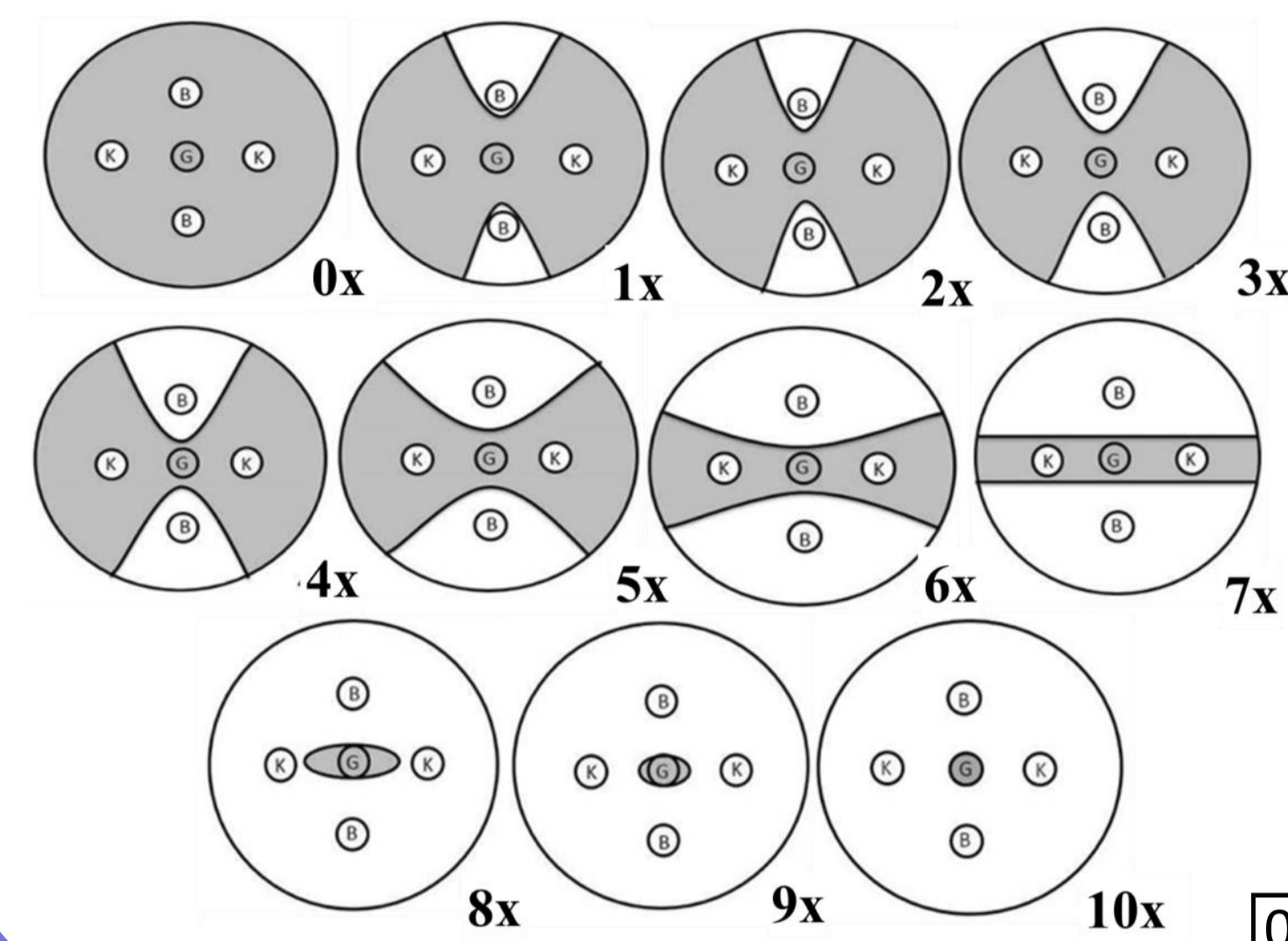
## Methods

Dual inhibition assays on agar medium in Petri dishes were performed to test for the inhibitory effect of liquid bacterial cultures and their cell free culture filtrates on the fungal growth.



Liquid bacterial cultures were grown in various culture media. (KB- King B broth, TSB- tryptone soy broth, YG - yeast extract glucose broth, PDB - potato dextrose broth) with or without shaking for 3 days at 28°C.

Culture filtrates were prepared by filter sterilization of the culture supernatants and with addition of phenylmethylsulfonyl fluoride (1mmol final concentration).



Sizes and shapes of inhibition zones were evaluated according to a 11- degree scale of the inhibitory effect (N. Pék 2015).

gray: mycelial lawn

0x: none inhibition

1-2x: weak inhibition

3-4x: moderate inhibition

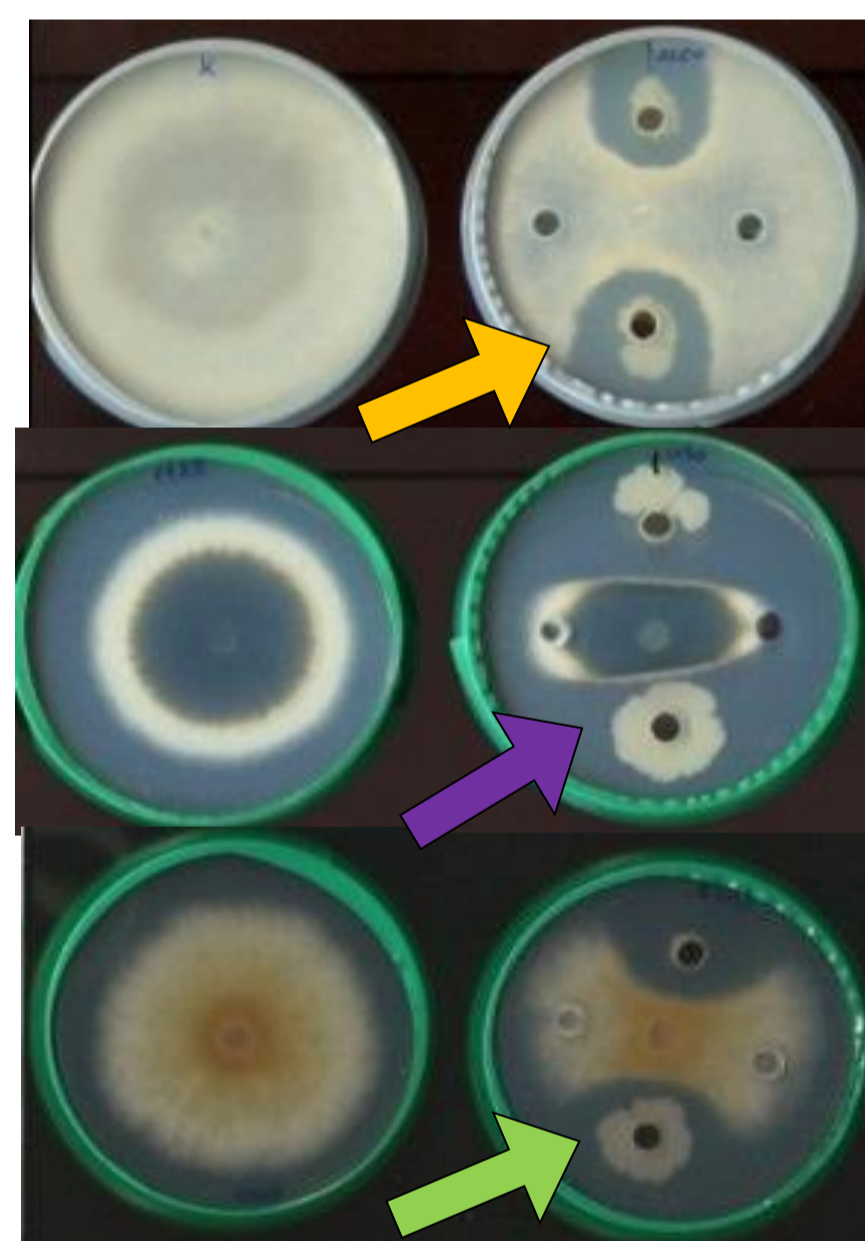
5x-: strong inhibition



## Inhibitory effect of *Bacillus subtilis*

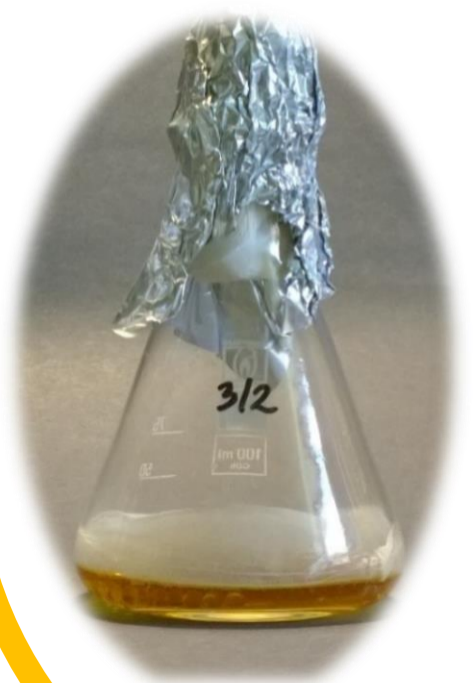
Both shaken and non-shaked cultures of *B. subtilis* exhibited strong/moderate and long term (21 day) inhibition on the growth of *S. sclerotiorum* (4-5x) and *A. alternata* (6-7 x) isolates. In contrast, much less inhibition zones were obtained against *F. graminearum* isolate (2-5 x). Inhibition (4-5x) could be observed only for a short period (7 days).

Fungal isolates	Incubation time (day)	Inhibitory effect of bacterial cultures							
		Shaked				Non shaked			
		PDB	TSB	KB	YG	PDB	TSB	KB	YG
<i>Sclerotinia sclerotiorum</i>	7	4x	5x	4x	4x	5x	5x	5x	5x
	14	4x	5x	4x	4x	5x	5x	5x	4x
	21	4x	5x	4x	4x	5x	5x	5x	4x
<i>Alternaria alternata</i>	7	7x	7x	7x	7x	7x	7x	7x	7x
	14	7x	7x	7x	7x	7x	7x	7x	7x
	21	6x	7x	7x	7x	6x	7x	7x	7x
<i>Fusarium graminearum</i>	7	4x	5x	5x	5x	5x	5x	5x	5x
	14	3x	5x	3x	3x	4x	4x	3x	4x
	21	2x	5x	2x	2x	2x	4x	3x	4x



(KB- King B broth, TSB- tryptone soy broth, YG - yeast extract glucose broth, PDB - potato dextrose broth)

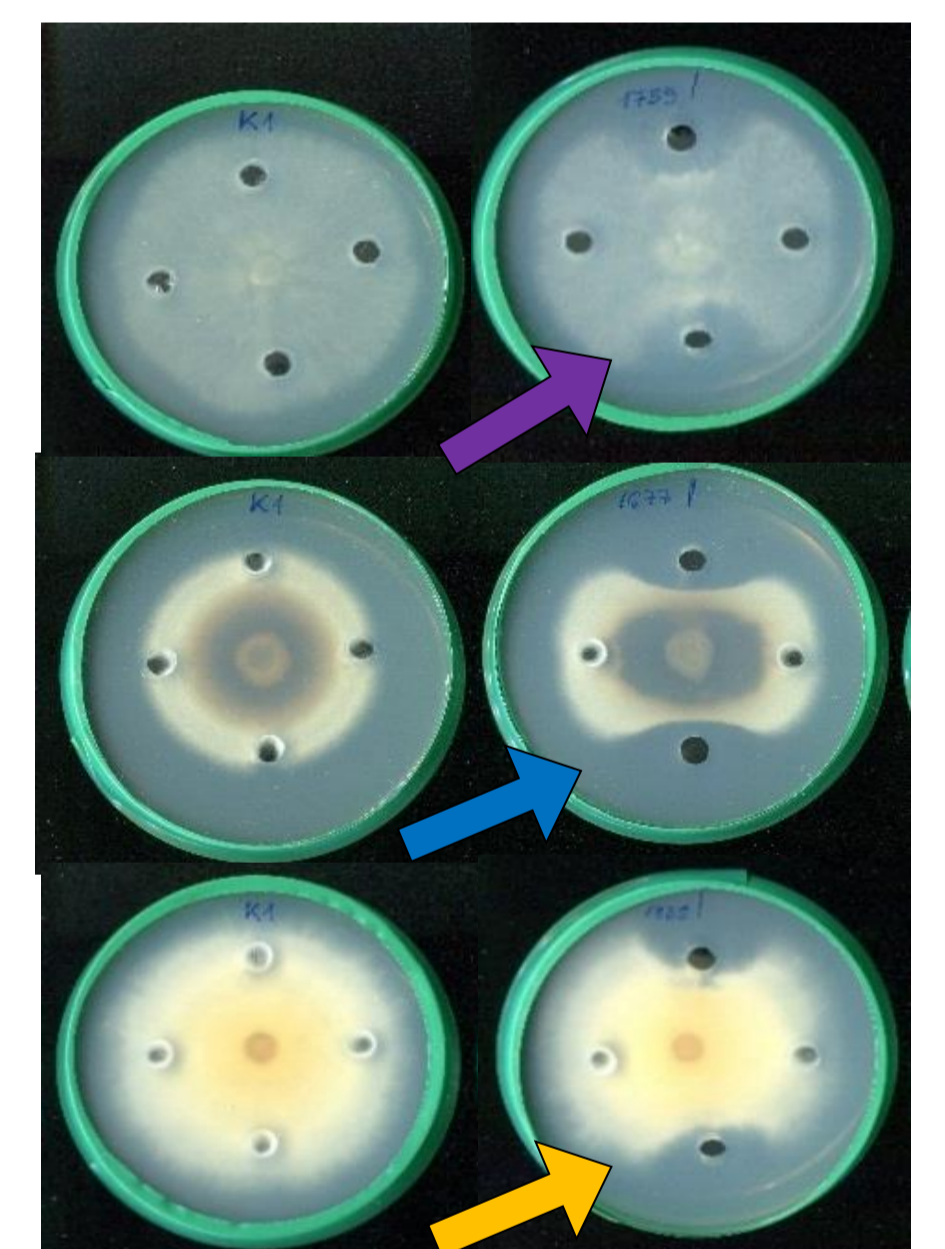
Under culturing conditions without shaking a skin-like film layer arose on the surface of the cultures when they were grown in TSB and KB media. This film production did not affected the antagonistic effect of the cultures: almost equal inhibition zones appeared by the PDB, TSB, KB and YG cultures against all three fungi. Other publication (Rahman et al. 2006) showed increased iturin concentration in non-shaked biofilm producing *B. subtilis* cultures, but the inhibitory effect of the cultures was not tested.



Less pronounced inhibition was observed by *B. subtilis* culture filtrates than that was obtained by the adequate cultures. Maybe inhibitory compounds were partially degraded and/or removed during the filtration of the cultures.

Fungal isolates	Incubation time (day)	Inhibitory effect of bacterial cultures							
		Non shaked cultures				Filtrate			
		PDB	TSB	KB	YG	PDB	TSB	KB	YG
<i>Sclerotinia sclerotiorum</i>	4	5x	5x	5x	5x	3x	3x	4x	1x
	7	5x	5x	5x	5x	0x	0x	1x	1x
	14	5x	5x	5x	4x	0x	0x	1x	1x
	21	5x	5x	5x	4x	0x	0x	1x	0x
<i>Alternaria alternata</i>	4	7x	7x	7x	7x	7x	5x	7x	4x
	7	7x	7x	7x	7x	4x	1x	6x	4x
	14	7x	7x	7x	7x	1x	0x	4x	3x
	21	6x	7x	7x	7x	1x	0x	4x	3x
<i>Fusarium graminearum</i>	4	5x	5x	5x	5x	4x	3x	4x	4x
	7	5x	5x	5x	5x	1x	1x	1x	1x
	14	4x	4x	3x	4x	1x	0x	1x	1x
	21	2x	4x	3x	4x	1x	0x	1x	1x

(KB- King B broth, TSB- tryptone soy broth, YG - yeast extract glucose broth, PDB - potato dextrose broth)



Inhibition zones by the culture filtrates were solely obtained against *A. alternata* isolate and only when the bacterial cultures were grown in King B (KB) and yeast extract glucose (YG) media. These filtrates showed long term, strong or moderate antagonistic effects (6-3x). Interestingly, the inhibitory effect was completely lost against *F. graminearum* and *S. sclerotiorum* isolates.

## Summary

Liquid cultures of *B. subtilis* strain were able to inhibit the growth of all three fungi, but in various extent. Scale of the inhibition was affected by the culture medium composition and mode of culturing: peptone based media (TSB, KB) and culturing without shaking elicited more inhibitory effect. Cell-free culture filtrates showed less intense antagonistic effects.

Regarding our results, the *B. subtilis* strain in liquid culture form might be a good candidate for the control of *F. graminearum*, *S. sclerotiorum*, and *A. alternata* in field conditions.