Bacillus licheniformis (ATCC 14580) inhibitory activity against opportunistic fungi

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Hypothesis:

- *B. licheniformis* (ATCC 14580) will exhibit antifungal properties by inhibiting mycelial development when exposed to specific opportunistic fungi as a filtrate.

Introduction:

•The common soil-dwelling endophytic bacterium *B. licheniformis*, strain CHM1, has been shown to inhibit growth from several pathogenic fungi. Phytopathogenic fungi (i.e. *Fusarium oxysporum*, *Phytophtora infestans*, *Penicillium digitatum*, etc...) possess the capability to infect and cripple small to large-scale agricultural crop harvesting operations (Wang et al 2009). The development of preventative methodologies and techniques are vital in order to suppress and contain phytopathogenic fungi within agricultural operations. Without preventative methodologies coupled with increasing global populations, agricultural fungal outbreaks could result in local to national-scale economic inflation and hardships.

Materials:

Bacterial Species:

- *B. licheniformis* (ATCC 14580)

Fungi:

- *Alternaria alternata* (Opportunist)
- *Rhizopus stolonifer* (Opportunist)
- *Penicillium chrysogenum* (Opportunist)
- *Mucor hiemalis* (Pathogen)

- Fungi were cultured on Sabouraud Dextrose Agar (SDA)
- Bacterium was reconstituted using a nutrient broth and then cultured on Trypticase Soy Agar (TSA).
- Luria Broth (LB) for suspension preparation

Suspension:

- **Growth Media:** The culture suspension of ATCC 14580 was prepared in LB and incubated at 30°C for 24 hours at 170 rmps. A sterile suspension was obtained by heating to 121°C for 30 minutes. Both suspensions were further centrifuged for 5 minutes at 5000 rmps. Supernatant was then collected.

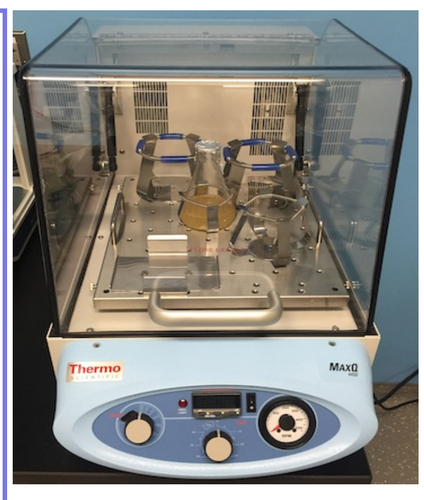

Fig. 1: Orbital Shaker with suspension of ATCC 14580.

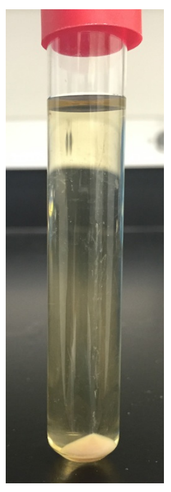

Fig 2: Supernatant with pellet.

Treatments:

In vitro:

- ATCC 14580 with *M. hiemalis* on PDA
- ATCC 14580 with *R. stolonifer* on PDA
- ATCC 14580 with *A. alternata* on PDA
- ATCC 14580 with *P. chrysogenum* on PDA

Fig. 3: Culture of ATCC 14580 on TSA

Methods:

•2mL of supernatant was spread on a plate containing PDA. Mycelia plug (5.0 mm diameter) was extracted using a sterile test tube and placed on PDA plate containing either sterile or unsterile culture supernatant. Colonies of fungi ranged in age at the time of mycelia plug extraction: 5 day, 2 day, 5 day, 5 day, in respect to 'Treatments' section from top to bottom. This was performed in triplicate for each fungal species and sterile/unsterile superantant for a total of 24 plates. Plates were incubated at 27°C for 48 hours.

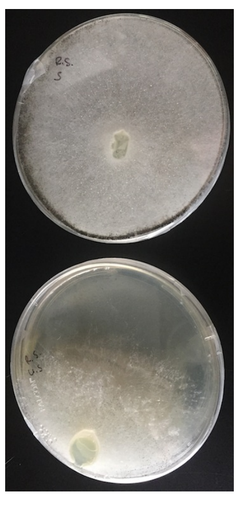

Fig. 4: *R. stolonifer* after 48 hours. Illustrates complete almost ineffectiveness. Note lesser number of fruiting bodies in unsterile (bottom) than sterile supernatant (top).

Fig. 5: EBA 21 Hettich Zentriguen was used for separating the supernatant from the pellet at 5,000 rmps for 5 minutes.

Fig. 6: Gram stain of ATCC 14580. Note gram-positive rod shape.

Fig. 7: Spore stain of ATCC 14580. Green pigmentation is endospore.

Fig. 8: *P. chrysogenum* (5 day) cultured on SDA.

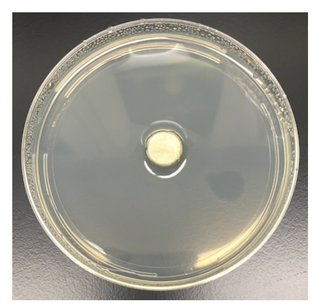

Fig. 9: Mycelia plug of *M. hiemalis* in ATCC 14580 supernatant on PDA.

Results:

Table 1: Oneway Analysis of Radial Growth over 24 hours (mm)

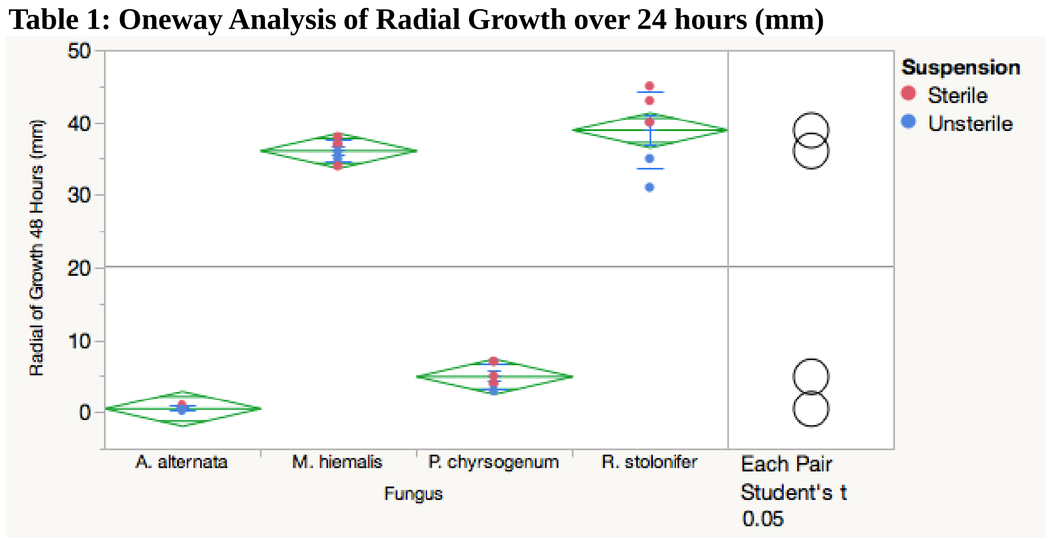

Table 1: Prob>F= <0.0001 Illustrates the radial growth of fungi in the presence of ATCC 14580 supernatant. The supernatant had the greatest effect on mycelial growth against *A. alternata* and *P. chrysogenum*.

Table 2: LSD Threshold Matrix and Ordered Differences Report of Table 1.

LSD Threshold Matrix

Abs(Dif)-LSD

	M. hiemalis	R. stolonifer	P. chyrsogenum	A. alternata
M. hiemalis	-1.0473	-0.4973	2.0194	2.3027
R. stolonifer	-0.4973	-1.0473	1.4694	1.7527
P. chyrsogenum	2.0194	1.4694	-1.0473	-0.7639
A. alternata	2.3027	1.7527	-0.7639	-1.0473

Positive values show pairs of means that are significantly different.

Table 2. Further illustrates the effect of ATCC 14580 on fungi. Note positive values under LSD Threshold Matrix represents means that are significantly different from each other after 24 hours of exposure.

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
M. hiemalis	A. alternata	3.350000	0.5020513	2.30274	4.397261	<.0001*
M. hiemalis	P. chyrsogenum	3.066667	0.5020513	2.01941	4.113927	<.0001*
R. stolonifer	A. alternata	2.800000	0.5020513	1.75274	3.847261	<.0001*
R. stolonifer	P. chyrsogenum	2.516667	0.5020513	1.46941	3.563927	<.0001*
M. hiemalis	R. stolonifer	0.550000	0.5020513	-0.49726	1.597261	0.2863
P. chyrsogenum	A. alternata	0.283333	0.5020513	-0.76393	1.330594	0.5788

Table 3: Oneway Analysis of Radial Growth over 48 hours (mm)

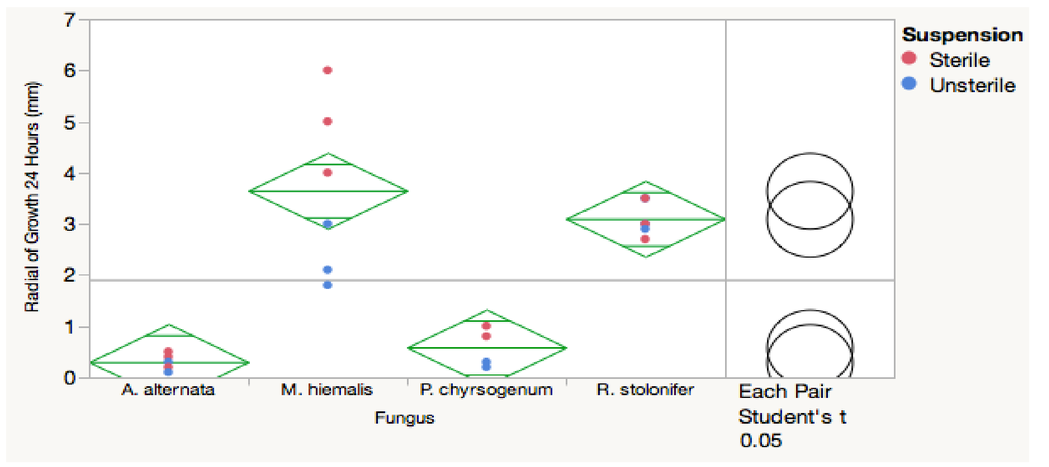

Table 3: Prob>F= <0.0001 Illustrates the radial growth of fungi in the presence of ATCC 14580 supernatant. After 48 hours, the supernatant only had a truly positive effect was *A. alternata*.

Conclusion:

•The sterile and unsterile suspension proved ineffective against *R. stolonifer* and *M. hiemalis* for both inhibition of mycelial growth and new colony formation.
•The sterile and unsterile suspension did inhibit the overall growth of *P. chyrsongenum* and *A. alternata*. However, it not prevent the formation of new colonies of either species.
•ATCC 14580 does possess antifungal properties. However, ATCC 14580 proved ineffective on fast sporulating fungi (i.e. *R. stolonifer*).
•Important to take note; ATCC 14580 supernatant did not inhibit new colonies of *R. stolonifer*, *P. chrysogenum* and *M. hiemalis*.
•*In vitro* studies done on PDA where ATCC 14580 and one of the four fungi were spread. 'Dead zones' were observed between ATCC 14580 and *R. stolonifer*, *M. hiemalis* and *A. alternata*. R. stolonifer also did not produce fruiting bodies over a 2 week period. Therefore at higher concentrations, ATCC 14580 could have a greater effect on fungal growth.

Acknowledgements:

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References:

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