Production and Application of Syringomycin E as an Organic-Compatible Agrifungicide

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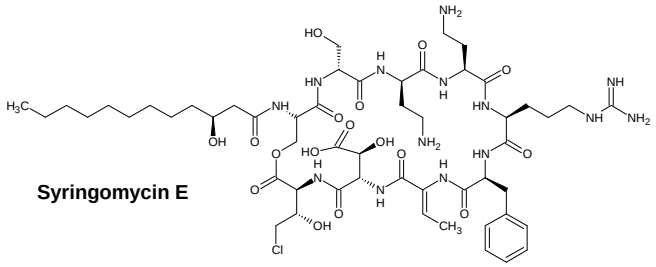
Abstract

Regulatory exclusion of synthetic chemicals limits the options for disease control in organic farming. Syringomycin E (SRE) is a cyclic lipodepsipeptide produced by the plant-associated bacterium *Pseudomonas syringae*. This natural product is lethal to a broad range of fungi at concentrations below 10 µg•ml^{-1} and is not antibacterial or toxic to animal cells. As a natural product, SRE was expected to be an organic-compatible agrifungicide. An enhanced SRE producer strain of *P. syringae*, G10, was obtained by ultraviolet mutagenesis of the conventional producer strain B301D. G10 was grown in a 10 l-capacity Winpact Bioreactor and Fermenter system in a medium containing 1% glucose, 1% mannitol, 0.4% histidine, 0.8 mM $MgSO_4$, and 0.01 mM $FeCl_3$ in 0.8 mM potassium phosphate at pH 6.7, 28℃, and with 350 rpm stirring. The SRE yield reached 50 mg•l^{-1} in 40 h. SRE in the culture broth was purified using an ÄKTA avant 150 liquid chromatography system with a C18 column (RPC-3, 6.4 × 100 mm). Isopropanol and formic acid were used as solvents to meet organic standards specified by the United States Department of Agriculture. The product SRE inhibited growth of *Pythium ultimum* oospores at 10 µg•ml^{-1} *in vitro*. Organic cucumber seeds with or without SRE coating (5-10 µg SRE per seed) were placed on water agar and on farm soil naturally contaminated with *Pythium* species. There was no significant difference between germination rates of SRE-coated seeds and non-coated seeds on water agar. SRE-coated cucumber seeds germinated at the rate of 70% on naturally infested soil while all of non-coated seeds were killed. This study invented a novel production method of organic-compatible SRE and demonstrated its potential as an agrifungicide.



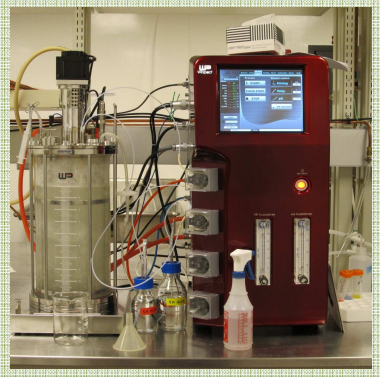
Syringomycin E

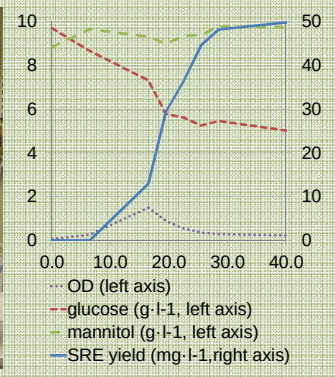
Introduction

P. ultimum is a soil-borne pathogen causes damping-off of seedlings. The losses to field- and greenhouse-grown cucurbits are severe. Even though seed treatment is one of the most inexpensive and effective way to protect plants, regulatory exclusion of synthetic chemicals makes disease control difficult. Antifungal activity of SRE has been reported against many phytopathogens including *Pythium, Fusarium,* and *Rhizoctonia* species. An organic-compatible production method of SRE and an use of SRE for organic treatments of cucumber seeds were pursued.

Results

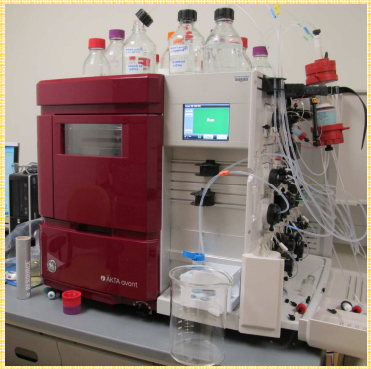
Fermentative production of SRE

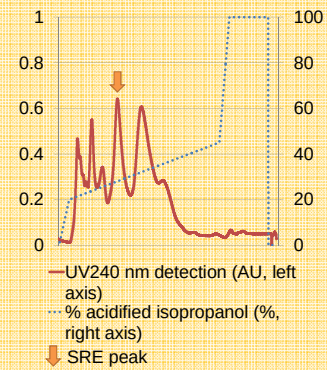




A new strain of *P.* syringae, G10, was isolated from ultraviolet mutagenesis of B301D. Fermentative growth of G10 produced 50 mg•l^{-1} of SRE in 40 h

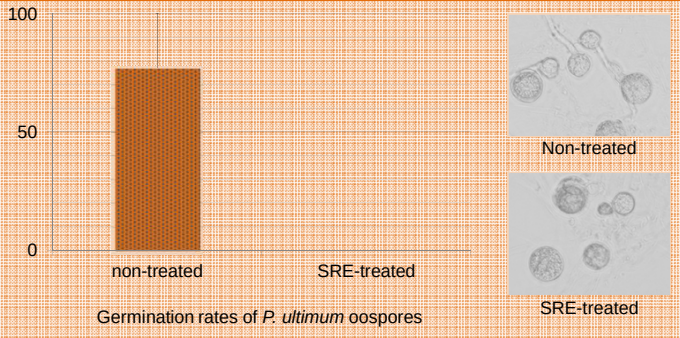
Organic-compatible purification





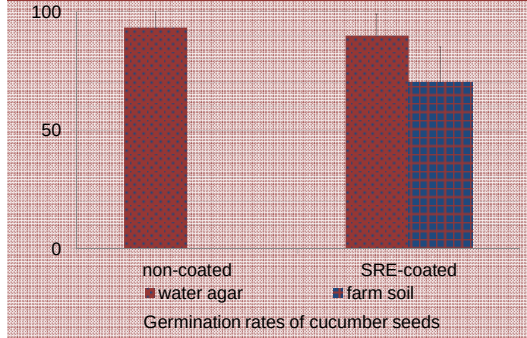
The SRE peak was detected with a wavelength of 240 nm. SRE was eluted with 26-30% isopropanol acidified with 0.1% formic acid.

Inhibition of *P. ultimum*



The germination rate of SRE-treated oospores of *P. ultimum* was 0% (n=26) though 71% (n=31) of non-treated oospores was germinated.

Protection of seeds without phytotoxicity



On water agar, non- and SRE-coated seeds had germination rates of 93% (n=30) and 90% (n=30), respectively. SRE-coated cucumber seeds germinated at the rate of 70% (n=23) on naturally infested soil while non-coated seeds had a germination rate of 0% (n=23).

Conclusions

A scalable and organic-compatible method of SRE production was invented. The product when used as a seed treatment inhibited the development of *Pythium* disease and did not influence the germination of organic cucumber seeds. SRE is a potential organic-compatible agrifungicide.

Acknowledgements

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