Degradation of Salicylate, an Important Plant-Signaling Molecule, by the Fungal Plant Pathogen *Sclerotinia sclerotiorum*

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Introduction

Sclerotinia sclerotiorum is a nectrotrophic phytopathogen (**Figure 1**) that has the ability to infect up to 406 plant species, including many cash crops [6]. The plant pathogen is capable of significantly reducing crop yields as it has been estimated that annually it accounts for $200 billion losses in the United States alone [2]. The key pathogenic trait of *S. sclerotiorum* is its ability to synthesize oxalate (toxin) [1]. It has been proposed that isolates of *S. sclerotiorum* that produce more oxalate tend to be more pathogenic than strains that produce less [4].

One circumstance that has been shown to regulate oxalate production by the organism is the type of carbon sources available in the environment [4]. External pH and buffering capacity of the environment also plays a large role in oxalate production by *S. sclerotiorum* [3]. It has been suggested from past studies that increasing the culture pH or buffering capacity of the medium, increases the ability of the organism to produce oxalate [3].

Salicylate is a plant-signaling molecule synthesized by plants in response to a pathogen attack and is involved in activating plant defenses. The compound plays a role in systematic acquired resistance (SAR), which is the process by which older, infected leaves causes the development of resistance in younger leaves [6]. Very little work has been done to determine whether *S. sclerotiorum* has the ability to metabolize salicylate, and if it does, what effect it has on the metabolism and growth of the organism.

Goals

- To determine whether salicylate was degraded by *S. sclerotiorum*
- To determine the impact of salicylate on biomass formation, oxalate production, and culture acidification by *S. sclerotiorum*

Methods and Materials

Growth of Culture. *S. sclerotiorum* isolates (D-E7, 105, and W-15HT) were maintained on Potato Dextrose Agar (PDA) plates at 25°C and transferred (5 mm plug) to 50 mL of culture medium. Culture medium (pH 6.5) consisted of minerals and trace metals supplemented by 0.1% soytone, 50 mM MES (buffer), glucose, and salicylate. Cultures were incubated for 7 days while shaking at 200 rpm. After the 7 days of incubation the cultures were pulled from the incubator and harvested.

Biomass (Growth) Determination. Biomass was harvested by the use of a vacuum system and captured by Whatman® 70 mm filter paper. The biomass was then dried at 50°C for 72 hours, cooled in a desiccator, and then weighed with the use of an electronic scale.

Substrate Level Determination. Filtrate was separated from growth by use of filter paper. The pH of the filtrate was determined with the use of an Orion model 230A pH meter and an Orion semi-micro combination electrode. A 1 mL sample was also collected for analysis by a Beckman Gold high performance liquid chromatograph fitted with a 300 mm Bio-Rad Aminex HPX-87H column. Oxalate and salicylate detections were done at 210 nm and the glucose was detected by the refractive index detector. All concentrations of the compounds were expressed on the millimolar (mM) basis. Throughout the study, no distinctions were made between organic acids and their salt forms.

Results

- Salicylate was degraded by all three isolates of *S. sclerotiorum*; however, salicylate was not supportive **(Table 1).**
- Uninoculated controls showed that salicylate was not abiotically degraded under culture conditions (data not shown). Also, salicylate was not synthesized by *S. sclerotiorum* during growth in the medium **(Table 1)**.
- Salicylate was not growth supportive and at higher concentration (10 mM) seemed to inhibit growth **(Table 1)**.

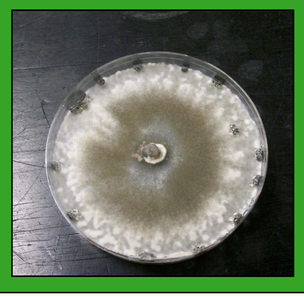

Figure 1. *S. sclerotiorum* D-E7 on PDA plate

Table 1. Salicylate degradation and biomass accumulation by *S. sclerotiorum* isolates D-E7, 105, and W-15HT.

	Salicylate Degradation (mM)			Biomass Produced (g)		
Addition	D-E7	105	W-15HT	D-E7	105	W-15HT
None	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.020 ± 0.002	0.009 ± 0.001	0.017 ± 0.001
1 mM Salicylate	0.24 ± 0.05	0.75 ± 0.05	0.00 ± 0.00	0.022 ± 0.004	0.006 ± 0.003	0.015 ± 0.002
10 mM Salicylate	6.05 ± 1.62	9.04 ± 0.27	3.14 ± 0.98	0.029 ± 0.004	0.012 ± 0.002	0.025 ± 0.004
25 mM Glucose	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.137 ± 0.002	0.061 ± 0.028	0.138 ± 0.068
25 mM Glucose + 1 mM Salicylate	0.18 ± 0.30	0.00 ± 0.00	0.00 ± 0.00	0.125 ± 0.007	0.057 ± 0.003	0.117 ± 0.018
25 mM Glucose + 10 mM Salicylate	2.95 ± 3.90	7.09 ± 1.05	2.50 ± 3.76	0.087 ± 0.002	0.030 ± 0.003	0.066 ± 0.025

- Salicylate did not enhance nor repress oxalate production in the presence of glucose **(Table 2)**.
- Salicylate did not enhance nor repress the acidification of the medium **(Table 2)**.

Table 2. Effect of salicylate degradation on biomass and oxalate produced, glucose consumption, and acidification of the medium by *S. sclerotiorum* D-E7.

Addition	Salicylate (mM)	Biomass (g)	Oxalate (mM)	Glucose (mM)	Final pH
None	0.00 ± 0.00	0.020 ± 0.002	3.24 ± 0.22	0.00 ± 0.00	6.46 ± 0.02
1 mM Salicylate	0.24 ± 0.05	0.022 ± 0.004	4.33 ± 0.18	0.00 ± 0.00	6.39 ± 0.02
10 mM Salicylate	6.05 ± 1.62	0.029 ± 0.004	7.83 ± 1.33	0.00 ± 0.00	6.24 ± 0.07
25 mM Glucose	0.00 ± 0.00	0.137 ± 0.002	11.45 ± 0.18	0.00 ± 0.00	5.26 ± 0.04
25 mM Glucose + 1 mM Salicylate	0.18 ± 0.30	0.125 ± 0.007	9.81 ± 2.17	1.29 ± 2.23	5.24 ± 0.11
25 mM Glucose + 10 mM Salicylate	2.95 ± 3.90	0.087 ± 0.002	12.84 ± 2.50	1.58 ± 2.74	4.92 ± 0.21

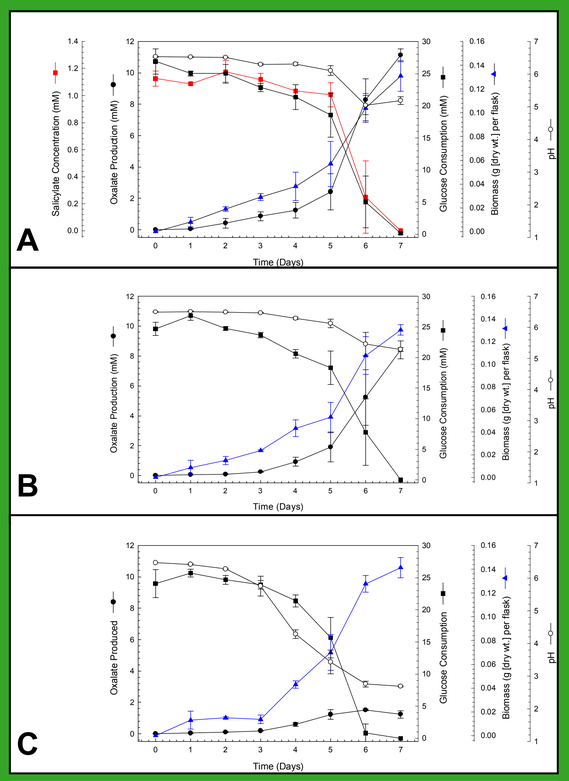

Figure 2. Time course analysis of growth, oxalate accumulation, glucose and salicylate consumption, and culture pH for *S. sclerotiorum* D-E7 growth in medium containing 0.1% soytone, 50 mM MES, 25 mM glucose, and 1 mM salicylate (A); 0.1% soytone, 50 mM MES, and 25 mM glucose (B); and 0.1% soytone and 25 mM glucose (C).

- Salicylate in the culture medium does not repress growth or oxalate synthesis of *S. sclerotiorum* **(Figure 2)**.
- All glucose and salicylate is consumed during time course, and each correlate positively which the growth of the organism and the production of oxalate **(Figure 2)**.
- From HPLC analysis of the time course study, potential intermediates from the degradation of salicylate were observed (data not shown).

Summary

Many microorganisms have the ability to degrade salicylate, including *Streptomyces*, *Pseudomonas*, and *Micrococcus* species [6]. It is still unknown whether this same pathway (**Figure 3**) is followed by *S. sclerotiorum*. However, the ability of *S. sclerotiorum* to degrade salicylate may allow this phytopathogen to shut down plant defenses by intercepting and degrading plant-signaling molecules that are directly linked with the activation of plant defenses.

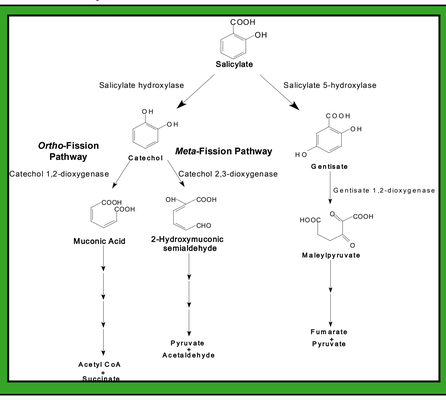

Figure 3. Possible pathway of salicylate degradation by *Sclerotinia sclerotiorum* that is observed in other microorganisms [6].

Literature Cited

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