The Effects of Jasmonate Derivatives on *Escherichia coli* Growth

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Abstract

Small cyclic plant signaling molecules called jasmonates serve as activators of wound response pathways and growth regulators in many types of plants; however, new connections are being made between these molecules and microbial life. This project focuses on how different jasmonate molecules can alter growth patterns in *E. coli*. K-12 W3110 derived strains' growth were measured by growth curve analysis in several jasmonate solutions. Five jasmonates were synthesized from methyl jasmonate, the most common naturally occurring jasmonate. Results indicate that methyl jasmonate and ethyl jasmonate caused a small dip in growth at about 6 hours, whereas jasmonic and jasmonolic acid had little to no effect on growth. Methyl jasmonol impeded growth as well, but did not show the same pattern as methyl jasmonate. Our ethanol control showed no major difference in growth. These results indicate that the presence of certain jasmonates directly affect bacterial growth rather than by some side pathway. Further research into the genetic changes that influence jasmonate resistance may shed light on mechanisms through which bacteria interact with plant hormones.

Introduction

- Jasmonates are a class of lipid signaling hormones generated by many biochemical response pathways in plants. Jasmonates are oxylipins, as they are derived via oxygenated polyunsaturated fatty acids, and are found in nearly all plant life.
- Jasmonates such as methyl jasmonate and jasmonic acid are used in complex biochemical pathways involved in wound response (Farmer & Ryan, 1990). Jasmonates interact with different kinase proteins to inhibit general plant growth and activate plant senescence (Reinbothe *et al.,* 2009).
- Previous research shows that MeJ treatment of A*rabidopsis thaliana* caused a significant change in the rhizobial microbe composition (Carvalhais *et al.,* 2013). This suggests that the activation of jasmonate signaling pathways may modulate bacterial growth.

Methods

- **Preparation of Jasmonate Analogs:** Five jasmonate analogs were prepared: methyl jasmonate (MeJ), jasmonic acid (JA), ethyl jasmonate (EtJ), methyl jasmonol (MeJOH), and jasmonolic acid (JAOH). 500 mg MeJ was used as starting material for each step of synthesis. See reaction scheme for synthesis details.
- **Analysis of Chemical Purity:** All products were analyzed using ^{1}H-NMR, ^{13}C-NMR, IR, GC-MS, and the OSU CCIC provided HRMS analysis.
- **Strain Reference / Media Preparation:** *E. coli* K-12 W3110 was used as the ancestral stain for the wild type and mutant strains used. LBK was prepared, and buffered to a pH of 7.0 using a 100 mM piperazine buffer. Any pH adjustments were made via either 5 M HCl or 5 M KOH (Creamer et al., 2017). 0.5 M stocks of each jasmonate isolate were prepared by dissolving each product in 100% ethanol.
- **Streaking of *E. coli* plates**: Frozen replicates of each strain were streaked onto an LBK agar plate, and were spread so that genetically isolated colonies could be extracted after incubation. Each plate was incubated for about 24 hours at 37 °C.
- ***E. coli* Overnights:** A small sample of an isolated colony of each strain was taken from the streaked plates and suspended in 2 ml of LBK media with no jasmonates. Overnights were placed in a rotating incubator for 16-18 hours at 37 °C.
- **Jasmonate Growth Curve Assays:** Sterile 96-well plates with 200 µL of LBK with either 0, 1, or 10 mM jasmonate, and 1uL of the strain being tested. OD_{600} was measured every 15 minutes over 22 hours.

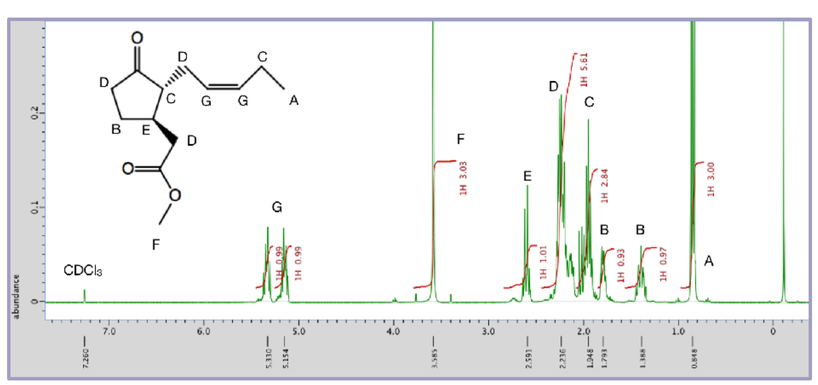
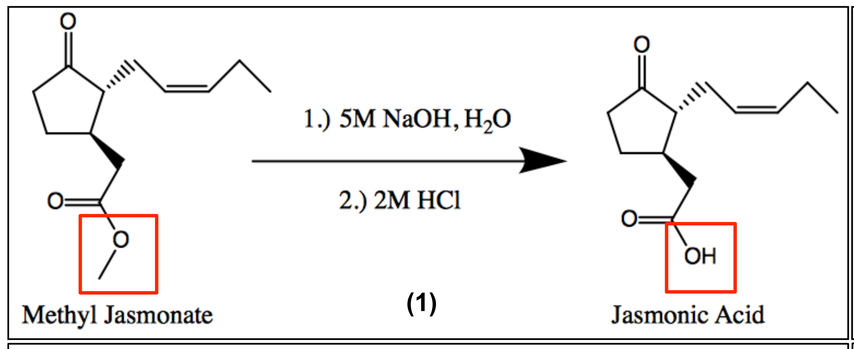
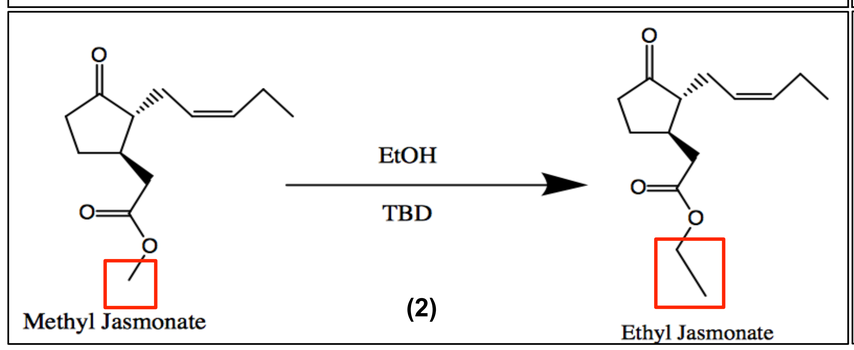


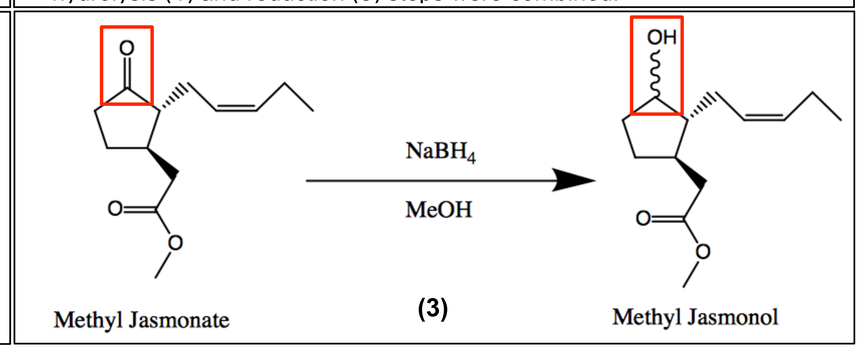
Figure 1: ^{1}H-NMR of Methyl Jasmonate

Jasmonate Preparation Reaction Scheme

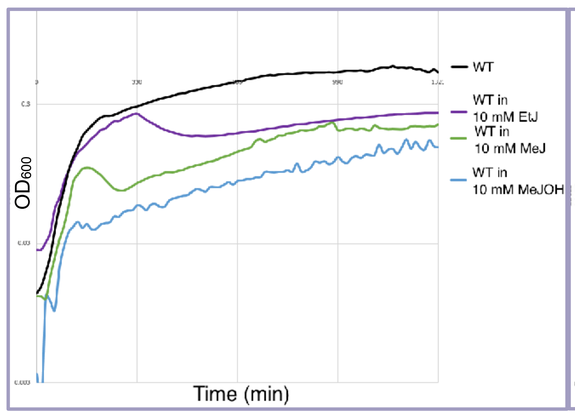


(1) Base Catalyzed Hydrolysis of methyl jasmonate to form jasmonic acid. 2.0 eq NaOH used to cleave terminal oxygen-methyl bond, aqueous product worked up with dilute HCl and washed with 3 x 40 ml $CHCl_3$.
(2) Transesterification of methyl jasmonate to form ethyl jasmonate. Catalytic triazabicyclodecene (TBD) and ethanol used to exchange methyl ester for ethyl ester. Washed with 2 x 10 ml 1M HCl, 10 ml brine, and 10 ml deionized H_2O to eliminate residual aqueous by-product.
(3) Reduction of methyl jasmonate to form methyl jasmonol. Excess methanol and 1.1 eq of sodium borohydride were used to reduce the ketone group. This lead to a racemic mixture of stereoisomers. Same acid wash as reaction (2). Similar setup used for preparation of jasmonolic acid, except hydrolysis (1) and reduction (3) steps were combined.





(2) MeJ, EtJ, and MeJOH Inhibit Growth



(3) JA, JAOH, and EtOH Show no Effect

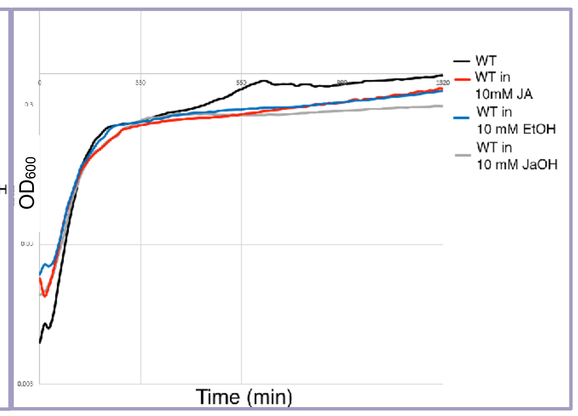
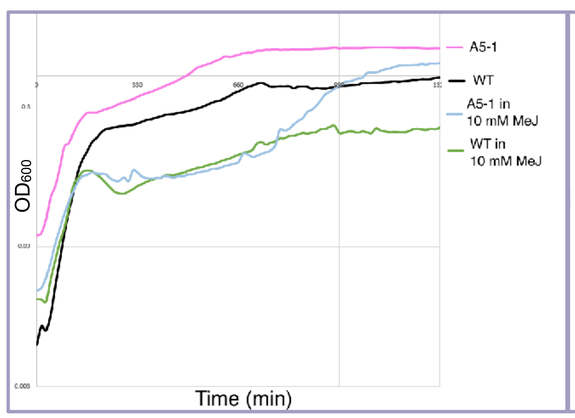


Figure 2: Both MeJ and EtJ show a small “dip” in growth around 300 - 500 min. MeJOH caused a large decrease in growth, but no dip.

Figure 3: Both JA and JAOH showed very little effect on growth in all strains tested. Ethanol control shows no difference from standard growth, indicating that the ethanol used to dissolve all products is inconsequential in overall growth.

(4) A5-1 Outgrows Wild Type



(5) *mdtE* Pump and *mar* Regulon Not Used

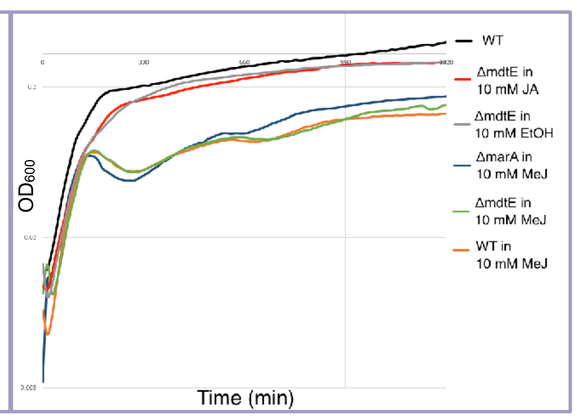


Figure 4: Some genetic difference in the A5-1 mutant strain allow for increased fitness under jasmonate stress. The *mar* regulon and the *mdtEF* pump are mutations of interest.

Figure 5: *mdtE* and *marA* knockout strains exhibit standard growth. Jasmonate resistance does not rely on the multi-drug efflux pump or the *mar* regulon

Conclusions

- Methyl jasmonate and ethyl jasmonate inhibit bacterial growth
  - Lowered endpoint growth in all strains tested
  - Dip in growth at about 5 hours
- Methyl jasmonol stress causes a larger decrease in growth
  - No dip observed
  - Ketone group may be responsible for dip in growth
- Jasmonic acid and jasmonolic acid do not affect growth
  - Ester-hydrolyzed jasmonate analogs likely not involved in mechanism that plants use to regulate bacterial growth
- Benzoate-evolved A5-1 strain has increased jasmonate tolerance
- *mdtE*, *mdtF*, *marA*, and *marR* knockouts grow similar to background strain
  - *mdtEF* pump and *mar* regulon not involved in jasmonate tolerance

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