

Volatiles produced by bacteria alleviate antagonistic effects of one associated fungus on *Dendroctonus valens* larvae

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Dear Editor,

Microbes play important roles in various symbiotic systems (Douglas, 2015; Xing et al., 2017; Zhu and Wu, 2016; Xu et al., 2016a). In the symbiosis formed by bark beetles and microbial associates, beetles formed symbiotic relationships with some fungi including mutualistic and antagonistic ones (Cheng et al., 2015; Lu et al., 2016; Scott et al., 2008), and these interactions, indirectly affected by a third or even fourth participator in a community context, may change from antagonistic ones to mutualistic ones. For example, associated bacterial volatiles can alleviate antagonistic effects from fungal associates on bark beetles (Wang et al., 2013; Zhou et al., 2016). However, key chemical signals that mediate the dynamic shift from antagonism to mutualism have received limited attention. Thus, this study tried to analyze these potential signals in this symbiosis.

Here, we hypothesized that volatiles derived from bacteria could inhibit the growth of associated fungus *O. minus*

and retain more carbohydrates for RTB larval growth. To prove that hypothesis, we first investigated whether bacterial volatiles could alleviate antagonistic effects of *O. minus* on the growth of RTB larvae. Secondly, we tested effects of bacterial volatiles on the growth of *O. minus* and its carbohydrate consumption. Thirdly, bacterial volatile profiling of three bacterial strains was analyzed. Lastly, effects of determinate bacterial volatiles on the growth of *O. minus* and its carbohydrate consumption were determined.

Results showed that *O. minus* significantly decreased the weight of RTB larvae (Figure 1A, $df=72.882$, $t=531.783$, $P=0.022$). However, bacterial volatiles alleviated the antagonistic effect from *O. minus* on bark beetle larvae (Figure 1B, one-way ANOVA, $F_{8,36}=52.780$, $P<0.001$). The weight of RTB larvae increased about 0.41, 0.75, 0.82, 1.41, 0.92, 1.16, 0.31, and 0.96 mg in phloem media colonized by *O. minus* in the presence of bacteria strains B301, B302, B904, B310, B317, B321, B322, and B911 respectively. However, the larval weight decreased about 3.0 mg in the absence of bacterial strains.

Further results revealed that the growth of *O. minus* was significantly inhibited by the eight bacterial strains (Figure 1C, one-way ANOVA, $F_{8,36}=52.780$, $P<0.001$).

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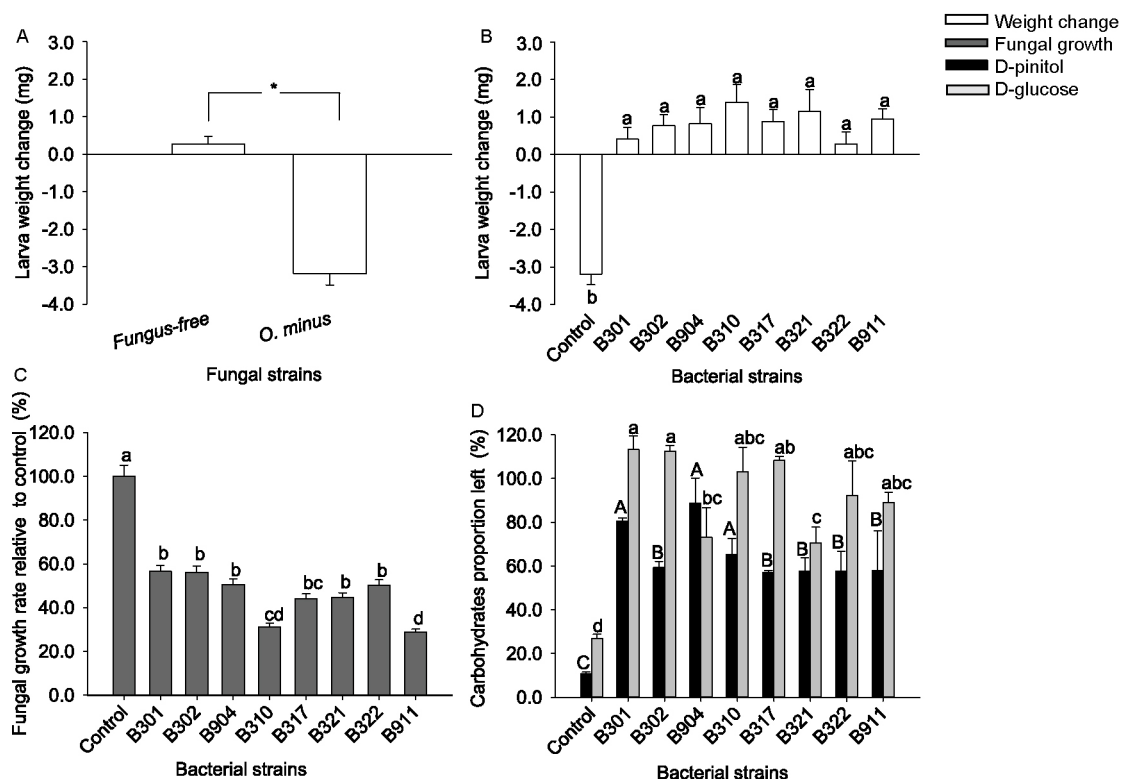


Figure 1 Average weight change of RTB larvae during a 6-day period after feeding on sterile medium and the medium with *O. minus* ($n=36$ for each treatment, A), average weight change of RTB larvae during a 6-day period after feeding on *O. minus*-free medium and *O. minus*-colonized medium in the presence of eight bacterial strains (*R. aquatilis* B301 B302 B904, *S. liquefaciens* B310 B317, and *Pseudomonas* sp. 7 B321 B322 B911, $n=40$ for each treatment, B), linear growth of *O. minus* (CMW25254) in the presence of eight bacterial strains ($n=5$ for each treatment, C), and carbohydrate consumption of *O. minus* (CMW25254) on phloem media in the presence of eight bacterial strains ($n=5$ for each treatment, D). The asterisk in (A) referred to significant difference analyzed by independent t test. Different letters in B–D above each bar referred to significant difference of multiple comparisons within each set of bars. (Capital letters for *D*-pinitol in (D), and lowercase letters for larval weight change in (B), fungal growth in (C) and *D*-glucose in (D)).

Growth rate of *O. minus* was decreased to less than 60% under effects of bacterial volatiles from selected strains. Besides, consumption of *D*-pinitol (one-way ANOVA, $F_{8,36}=28.608$, $P<0.001$) and *D*-glucose (one-way ANOVA, $F_{8,36}=8.974$, $P<0.001$) were significantly reduced by these bacterial strains (Figure 1D). There was about 80.7%, 59.5%, 88.4%, 65.3%, 56.6%, 57.9%, 57.9%, and 57.6% of *D*-pinitol remaining in fungus-grown phloem media in the presence of bacterial strains B301, B302, B904, B310, B317, B321, B322, and B911 respectively. However, there was only about 10.1% of *D*-pinitol remaining in the control group. Similarly, there was about 113.4%, 112.7%, 73.6%, 103.4%, 108.6%, 70.7%, 92.8%, and 89.0% of *D*-glucose remaining in fungus-grown phloem media in the presence of the eight bacterial strains respectively. However, there was only about 27.4% of *D*-glucose remaining in the control group.

With SPME-GC-MS, 32 chemicals were identified. Particularly, 18 volatiles were detected from *R. aquatilis* B301 (Table S1 in Supporting Information), 21 from *S. liquefaciens* B310 (Table S2 in Supporting Information), and 19 from *Pseudomonas* sp. 7 B321 (Table S3 in Supporting Information). Nine chemicals, including ammonia, acetic acid, 3-methyl-1-butanol, 2-methyl-1-butanol, (*R*)-2-hex-

anol, 2-undecanone, 2-heptanone, 1,10-undecadiene, and 2-butanol were all detected in the three strains. Among them, 1,10-undecadiene and 2,2-dimethyl-hexane have a slight inhibitory effect on *O. minus* growth (Table S4 in Supporting Information). The compounds 2-butanol, 2-heptanone, 3-methyl-1-butanol, ammonia, and (*R*)-2-hexanol have a significant inhibitory effect on *O. minus* growth (Table S4 in Supporting Information). Thus, more carbohydrates were left for RTB larval growth.

This study demonstrated that seven dominant bacterial volatiles including 3-methyl-1-butanol, 1,10-undecadiene, 2-butanol, 2-heptanone, 2,2-dimethyl-hexane, (*R*)-2-hexanol, and ammonia were considered as candidate chemicals which affect growth and carbohydrate consumption of *O. minus*. Further results showed that these chemicals conspicuously inhibited the growth of *O. minus* to retain more unconsumed *D*-pinitol and *D*-glucose (Table S4 in Supporting Information).

Bacteria play important roles in improving bark beetle fitness (Xu et al, 2016b). Our results suggest that bacterial symbionts of bark beetles can indirectly benefit bark beetle larvae by inhibiting the growth and carbohydrate consumption of detrimental fungus. Our work reveals that the microbe-mi-

probe interactions may affect pair-wise interactions between the insect and its microbial associates. Further work needs to be conducted to test microbe-microbe interactions among broader taxonomic range of insect-microbe symbioses.

Compliance and ethics The author(s) declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Table S1 Volatiles detected from bacterial strain *R. aquatilis* B301 associated with *D. valens* larvae

Table S2 Volatiles derived from bacterial strain *S. liquefacien* B310 associated with *D. valens* larvae

Table S3 Volatiles derived from bacterial strain *Pseudomonas* sp. 7 B321 associated with *D. valens* larvae

Table S4 Effects of 7 bacterial volatiles on growth and carbohydrate consumption of *O. minus* (CMW25254)

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