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Impact of volatiles of the rhizobacteria *Serratia odorifera* on the moss *Physcomitrella patens*

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Volatiles are important infochemicals acting aboveground as well as belowground between organisms. In order to understand the complex volatile network of an entire ecosystem, the habitat at the border between the atmosphere and the soil has to be considered. Mosses are the dominant colonists of this habitat. Here we tested the reaction of the moss *Physcomitrella patens* upon exposure to rhizobacterial volatiles. In a closed test system, when CO_2 is a dominant component of the bacterial volatile mixture, *P. patens* growth was promoted, while in the natural-like open test system volatiles with negative influences possess their effects resulting in growth inhibitions of the moss. Growth retardation is less pronounced when the volatiles were applied in a later stage of development of the moss.

The typical smell in a forest is primarily due to volatile emission of bacteria. For example, the volatile terpenoid geosmin is emitted by Streptomyces species and is responsible for the characteristic earthy and muddy bouquet.[1,2] Many other bacteria contribute also to the scents in the atmosphere which can be recognized by various animals including humans and also by plants.[3] Volatiles are especially suitable to act as mediators between organisms because they can be detected in small quantities and can diffuse over long distances. They have a small molecular mass (less than 300 dalton) and a high vapor pressure (0.01 kPa or higher at 20°C), and tend to be lipophilic rather than hydrophilic. Together, these features support evaporation. These infochemicals do not only act aboveground, but diffuse into the air-filled pores of the apparent unsuitable or inappropriate soil.[4,5] Evidence increases that belowground volatile interactions are similarly complex as aboveground.[6-8] To understand the entire integrity of an ecosystem, volatiles in both habitats have to be considered.[9]

Mosses are small plants that live at the border of soil and atmosphere. They therefore might be appropriate test organisms to study volatile influences in this remarkable habitat. Bryophyta are one of the oldest groups of land plants which colonize diverse habitats.[10,11] Their life cycle is dominated by a photoautotrophic haploid gametophytic generation that supports a relatively simple and mainly heterotrophic diploid sporophyte. The haploid gametophyte appears in distinct developmental stages, the protonema and the gametophore or leafy shoot. Mosses are ideal model systems. They possess relative simple developmental patterns, they are suitable for cell lineage analysis, they facilitate genetic approches e.g., homologous recombination, and they respond similarly to plant growth factors and environmental cues as higher land plants.[12,13] *Physcomitrella patens* is a monoecious moss (i.e., both sex organs are present on the same individual) that can simply be grown in the lab and it was extensively studied in the past.

In order to study bacterial volatiles acting on *P. patens*, the moss was placed in one compartment of bipartite Petri dishes. After 10 days of incubation the other compartment was inoculated with *Serratia odorifera* 4Rx13 (2–3 x 10^7 cell forming units). In the control experiment no bacteria were applied. Only volatiles can diffuse

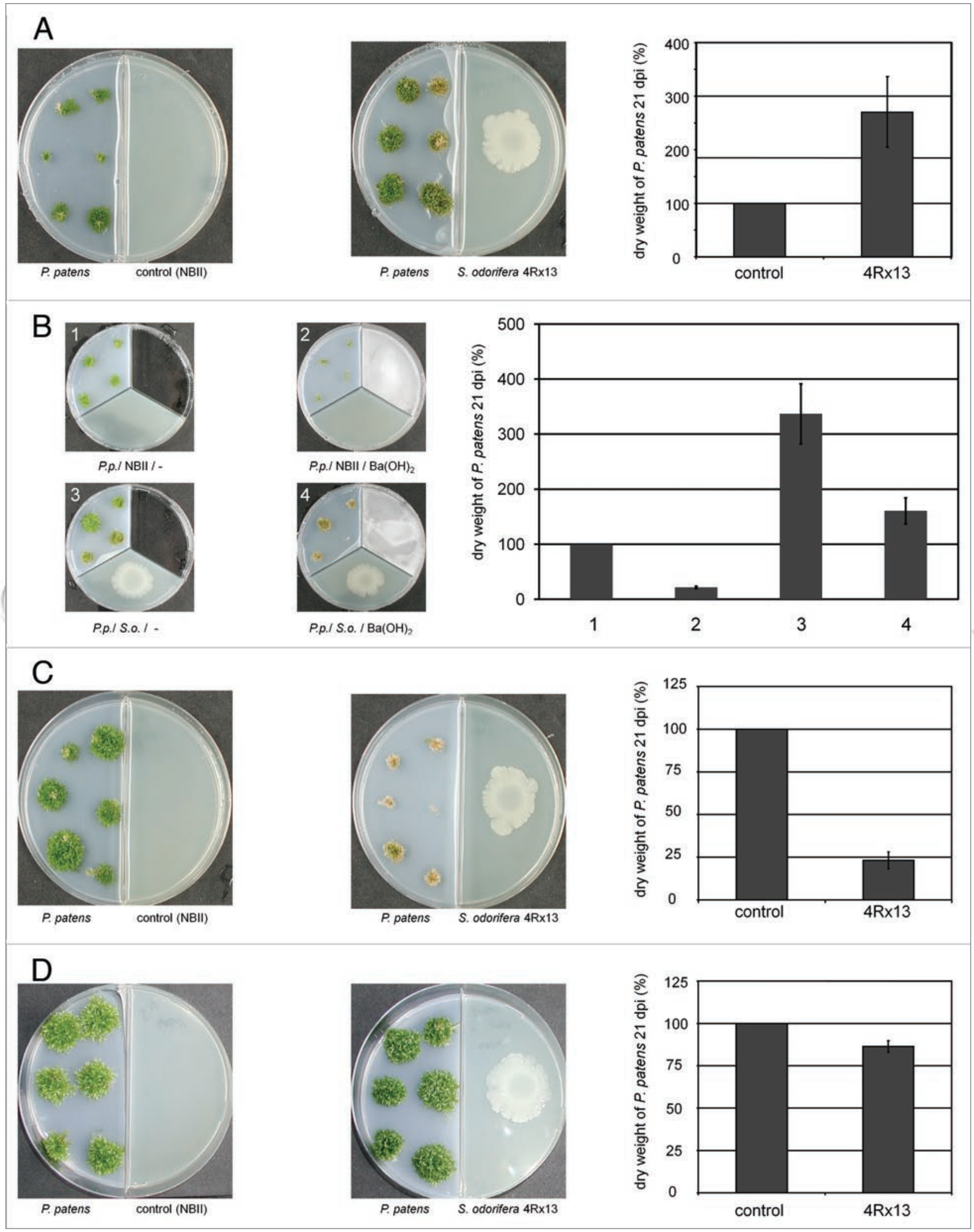

Figure 1. Co-cultivation of *Physcomitrella patens* with *Serratia odorifera* 4Rx13. (A) After three weeks of co-cultivation with *S. odorifera* in parafilm-sealed bipartite Petri dishes dry weight of *P. patens* was determined. The moss was 10 days old at the beginning of co-cultivation. Left: *P. patens*; middle: *P. patens* and *S. odorifera*; right: graphic presentation of the dry weight measurements. (B) Determination of the dry weight measurements in parafilm-sealed tripartite Petri dishes. Left: (1) *P. patens*, (2) *P. patens* and 0.1 mM $Ba(OH)_2$ solution, (3) *P. patens* and *S. odorifera*, (4) *P. patens, S. odorifera* and 0.1 mM $Ba(OH)_2$. Right: graphic presentation of *P. patens* dry weight measured in condition (1–4). (C) Determination of dry weight in non-sealed bipartite Petri dish. 10 day old *P. patens* were co-cultivated for three weeks with *S. odorifera.* (D) Determination of dry weight in non-sealed bipartite Petri dish. 19 day old *P. patens* were co-cultivated for three weeks with *S. odorifera.*

between the compartments. After three weeks of co-cultivation in parafilm-sealed Petri dishes, the dry weight of the moss was determined (**Fig. 1A**). Compared to the control experiment the moss grew much better during co-cultivation with the rhizobacteria, resulting in a 2.5 fold higher dry weight. This growth promotion is due to the accumulation of CO_2, since in a separated experiment it could be shown that the addition of $Ba(OH)_2$ captured CO_2 and consequently the CO_2 amount in the headspace of the Petri dish decreased. These lower CO_2 levels resulted in a growth reduction of *P. patens* (**Fig. 1B**; 1 vs. 2 and 3 vs. 4, respectively). A tenfold increase of CO_2 within one day due to the emission of the metabolic end product CO_2 of *S. odorifera* was previously determined in parafilm-sealed Petri dishes.[14] In an alternative approach using non-sealed Petri dishes ('open test system'), which also reflects the natural system more closely, CO_2 did not accumulate and ambient CO_2 concentrations were present in the Petri dish.[14] Co-cultivation of *P. patens* and *S. odorifera* under such conditions revealed strong growth inhibitions of *P. patens* reaching only 25% of the dry weight compared to the control (**Fig. 1C**). In another experiment the moss grew for 19 days in the Petri dish before the rhizobacteria were applied. In this case the growth of *P. patens* was only slightly inhibited (ca. 20%) (**Fig. 1D**). The comparison of the results of experiments presented in **Figure 1C vs. D** indicates that the strength of the volatile effects depends of the developmental stage of the moss.

These experiments showed that *P. patens* is a useful test organism, reacting to bacterial volatiles by growth alterations. Growth promotions were observed when CO_2 was the dominant volatile in the mixture, while under ambient CO_2 conditions the effects of inhibitory volatiles came to the fore. These growth inhibitions were more pronounced if young plants of the moss were fumigated by the bacteria. At present the individual components of the volatile mixture of *S. odorifera* are analysed which then will be applied individually to determine their bioactivity. Here, *P. patens* was successfully used as a volatile perceiving organism. In the future, it will also be of interest to study the ability of volatile emanation of moss.[8,15] Furthermore, to understand the volatile network at the border of the soil to atmosphere, more natural-like test systems have to be developed, in which the robust moss *P. patens* can be used as an indicator organism.

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