

Considering Microbial CO₂ during Microbe-Plant Cocultivation

Enhancement of plant growth by associated bacteria has been reported by multiple investigators (Lugtenberg and Kamilova, 2009). Many mechanisms have been demonstrated to play a role in this phenomenon. Recently, several reports have focused on the role of volatiles in beneficial microbe-plant interactions (Kanchiswamy et al., 2015; Schmidt et al., 2015). There are a number of thorough studies documenting that microbial volatile organic compounds (mVOCs) can influence plant growth, for example, by activating plant defenses and/or by inhibiting the growth of phytopathogenic microorganisms (Ryu et al., 2003; Groenhagen et al., 2013; Chung et al., 2016).

These studies used a variety of approaches to investigate the effects of microbial volatiles (Kai et al., 2016). Bi- and tripartite petri dishes, small containers in larger ones, top and bottom inoculated petri dishes, and dynamic airstream systems are frequently used experimental setups, which might in addition be sealed or unsealed. Particularly, sealed setups of microbe-plant cocultivations have to be evaluated carefully because it is important to consider that microorganisms simultaneously release complex mixtures of organic and inorganic volatiles. The latter include CO₂, HCN, NH₃, and H₂S, all of which possess dramatic impact on the growth of plants (Effmert et al., 2012). During cocultivation of microorganisms and plants in a sealed container, CO₂ concentrations may be elevated by microbial metabolic activity. For example, we measured up to 10-fold higher CO₂ concentrations in sealed petri dishes (Kai and Piechulla, 2009). Even when CO₂ concentrations in the headspace of the growth container are not elevated, the CO₂ emitted by microorganisms is likely to be taken up by the plant. Therefore, proper controls are needed in sealed experiments to eliminate the possibility that any growth-promoting effects are not solely due to microbial CO₂ or other inorganic volatiles emitted by the bacteria.

There are several approaches to avoid or control for microbial CO₂ (Kai and Piechulla, 2009; Piechulla and Schnitzler, 2016). One possibility is to ensure that ambient CO₂ levels are maintained in the experiments, for example, by scavenging CO₂ by carbonate formation using Ba(OH)₂ or similar. Depending on the trapping efficiency of Ba(OH)₂, CO₂ levels may be either reduced or eliminated completely from the system. A defined CO₂ level can be added thereafter. Alternatively, open systems can be used in which gas exchange is unhindered. Subsequent work should then include experiments to obtain VOC profiles and identify potentially bioactive mVOCs.

As more biologists investigate the mechanisms of growth promotion of plants by mVOCs, it is essential that researchers clearly describe the system that they

use—open or closed—and that they are able to show that the results they observed are indeed caused by mVOCs. It is unfortunate that such methodological issues are still often left unaddressed as the absence of this information undermines the scientific value of the publications. This is not a new concern—indeed, we need only revisit the history of experimental work surrounding ethylene to appreciate the impacts on scientific knowledge (Klassen and Bugbee, 2004; Wheeler, 2010; Van de Poel et al., 2015)—and it is one that the research community ignores to its own disadvantage. Without greater care, the potential of mVOCs to improve agriculture in the future cannot be properly assessed, and the consequences are sure to be missed opportunities to harness associated technologies for the benefit of all of us.

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