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# Sweet scents from good bacteria: Case studies on bacterial volatile compounds for plant growth and immunity

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**Abstract** Beneficial bacteria produce diverse chemical compounds that affect the behavior of other organisms including plants. Bacterial volatile compounds (BVCs) contribute to triggering plant immunity and promoting plant growth. Previous studies investigated changes in plant physiology caused by in vitro application of the identified volatile compounds or the BVC-emitting bacteria. This review collates new information on BVC-mediated plant-bacteria airborne interactions, addresses unresolved questions about the biological relevance of BVCs, and summarizes data on recently identified BVCs that improve plant growth or protection. Recent explorations of bacterial metabolic engineering to alter BVC production using heterologous or endogenous genes are introduced. Molecular genetic approaches can expand the BVC repertoire of beneficial bacteria to target additional beneficial effects, or simply boost the production level of naturally occurring BVCs. The effects of direct BVC application in soil are reviewed and evaluated for potential large-scale field and agricultural applications. Our review of recent BVC data indicates that BVCs have great potential to serve as effective biostimulants and bioprotectants even under open-field conditions.

**Keywords** Bacterial volatile compound · 2,3-butanediol · Induced systemic resistance · Plant growth-promoting rhizobacteria · Metabolic engineering

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## Introduction

Microbial metabolic activity includes the secretion of diverse infochemicals, and many of these secreted chemicals are volatile compounds (Audrain et al. 2015). Bacterial volatile compounds (BVCs) are characterized by low molecular mass (<300 Da), low boiling point, and high vapor pressure secreted from bacteria (Vespermann et al. 2007). Bacteria produce a wide range of volatile compounds that have beneficial or adverse effects on the growth and physiology of other organisms including plants, fungi, and other bacteria. Research during the past decade indicates that many bacteria species emit complex blends of volatile compounds (Effmert et al. 2012; Bitas et al. 2013; Audrain et al. 2015). For example, a blend of airborne chemicals emitted from specific beneficial bacterial group [called plant growth-promoting rhizobacteria (PGPR)] was discovered to trigger plant immunity and growth promotion in *Arabidopsis thaliana* seedlings (Ryu et al. 2003, 2004a, b). Since that first study, many volatile compounds with beneficial effects on plant growth and immunity have been identified in PGPR. A recent review proposed that these volatiles should be designated as BVCs rather than the other previously reported volatile organic compounds (VOCs) because bacterial volatiles include inorganic compounds such as ammonia, hydrogen sulfide, hydrogen cyanide, and nitric oxide (Bernier et al. 2011; Audrain et al. 2015).

This review analyzes recent case studies that investigate BVC effects on plant growth and immunity. We used three criteria for our analysis: (1) we focused primarily on studies conducted within the previous 5 years; (2) studies reporting the direct growth inhibition of a major fungal plant pathogen were eliminated due to previous reviews (Wheatley 2002; Effmert et al. 2012;

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Bailly and Weisskopf 2012; Weisskopf 2013) and (3) we did not include studies on BVC-induced systemic tolerance against abiotic stress because this information was recently discussed (Farag et al. 2013; Kanchiswamy et al. 2015). Instead, the present review discusses the following four topics: (1) new data of known BVCs showing their contribution to plant immunity and virulence of pathogenic bacteria; (2) newly identified BVCs that mediate beneficial effects on plant growth and immunity; (3) direct application of BVCs into soil which promotes plant growth and immunity; and (4) metabolic engineering of BVC biosynthetic pathways which can expand the scope of beneficial effects conferred by PGPR.

## New roles for acetoin and 2,3-butanediol

Earlier studies showed that plant growth and induced systemic resistance (ISR) were stimulated by the BVCs such as 2,3-butanediol and its precursor 2-hydroxy-2-butanone (acetoin) from specific PGPR strains (Ryu et al. 2003, 2004a, b) (Fig. 1). Many subsequent studies investigated similar C4 BVCs. Many plant-associated *Enterobacteriaceae* and *Firmicutes* utilize pyruvate (a final product of glucose fermentation by the Embden-Meyerhof pathway) to produce acetoin and 2,3-butanediol. In animals and plants, BVCs function as elicitors and suppressors of immune responses respectively (Hsieh et al. 2007; Rudrappa et al. 2010). A test of several 2,3-butanediol stereoisomers indicated that only (2R,3R)-2,3-butanediol promoted plant growth and induced resistance/tolerance (Ryu et al. 2004a, b; Han et al. 2006; Cho et al. 2008; D'Alessandro et al. 2014). Intriguingly some insects also utilize acetoin and 2,3-butanediol as pheromones and kairomones (Buttery and Ling 1984; Nout and Bartelt 1998; Moore and Moore 1999; Moore et al. 2002; Robacker and Lauzon 2002; Rochat et al. 2002; Bengtsson et al. 2009). Therefore, these BVCs also can be involved in the attraction of beneficial insects, which recognize the volatiles as pheromones or kairomones. In this session, we discuss the functions of 2,3-butanediol and acetoin in beneficial and pathogenic bacteria.

### Dual role of BVC-mediated interactions between bacteria and plantactions

Recent work showed that the plant pathogenic bacterium *Pectobacterium carotovorum* also produced the 2,3-butandiol same as a beneficial Gram-negative and -positive bacteria (Marquez-Villavicencio et al. 2011). Subsequent reports indicated that 2,3-butanediol was produced by other soft-rot causing plant pathogenic bacteria, such as *Dickeya* and *Pectobacterium* species as well. In this

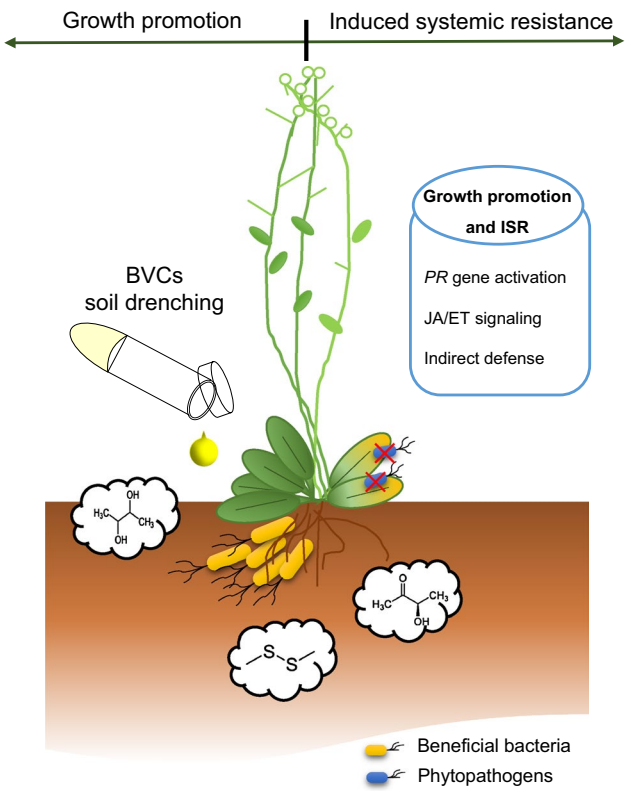


**Fig. 1** Role of bacterial volatile compounds emitted by plant growth-promoting rhizobacteria (PGPR). PGPR improve plant growth and enhance induced systemic resistance via BVCs. Soil application of BVCs enhances plant growth and immunity in the field. BVCs—2,3-butanediol, dimethyl disulfide, or acetoin are drenched into the soil. As a result of soil application, BVCs application conferred growth promotion and induced systemic resistance (ISR) through PR gene expression, jasmonic acid (JA)/ethylene (ET) signaling activation, and indirect defense against pathogens and insects. (Ryu et al. 2004a, b, 2013; Cortes-Barco et al. 2010b; Huang et al. 2012; Hahm et al. 2012; Song and Ryu 2013)

section, we discuss the function of 2,3-butanediol in pathogenic and beneficial bacteria, and examine its effects on plant physiology and induced immunity.

Acetoin and (2R,3R)-2,3-butanediol production primarily function in alkalinization of the growth environment, which protects bacteria from unfavorable acidic conditions (Xiao and Xu 2007). Mutagenesis of *budB* that confers 2,3-butanediol dehydrogenase/diacetyl reductase (product of the *budB* gene) to convert acetoin to (2R,3R)-2,3-butanediol and diacetyl to acetoin in plant-associated *Serratia plymuthica* significantly reduced bacterial growth on artificial medium compared with that of wild-type bacteria (Wevers et al. 2009).

2,3-butanediol modulates growth of soft-rot bacterial pathogens (*Pectobacterium* spp. and *Dicheya* spp.) and beneficial bacteria (*Serratia* spp.) (Kwan et al. 2013; Yu et al. 2000). The major virulence factors secreted by

soft-rot bacteria are plant cell wall degrading enzymes, including pectin lyases, cellulases, proteases, polygalacturonases, and pectinases. These enzymes require pH > 7 for full activity, whereas the plant apoplast generally has <pH 6 (Yu et al. 2000). Increase of pH in the plant apoplast is a major mechanism that leads to pathogenesis in *Pectobacterium* spp. and *Dickeya* spp. (Kwan et al. 2013). The 2,3-butanediol pathway has an important role during this step of pathogenic invasion because it alkalinizes the extracellular environment, and analysis of tissues showing soft-rot symptoms identified 2,3-butanediol (Effantin et al. 2011; Kwan et al. 2013). Beneficial plant bacteria such as *Serratia* and *Bacillus* produce large amounts of 2,3-butanediol under controlled conditions (Ryu et al. 2004a, b; Farag et al. 2006; Moons et al. 2011; Rao et al. 2012). Therefore, a question arises as to whether concurrent colonization of these two beneficial bacterial groups on plants could lead in some cases to greater virulence of pathogenic bacteria.

The initial study reporting that BVCs emitted by specific bacteria could suppress plant disease caused by pathogenic bacteria utilized *B. subtilis* as the emitter and *P. carotovorum* subsp. *carotovorum* as the target pathogen (Ryu et al. 2004a, b). This study indicated that the major BVCs emitted from *B. subtilis* were 2,3-butanediol and acetoin. Pretreatment with these compounds or bacteria for 1 week reduced the soft-rot symptoms caused by *P. carotovorum* subsp. *carotovorum*, whereas concurrent treatment did not elicit plant resistance (Ryu et al. 2004a, b). The authors proposed that BVC pretreatment enabled the plant to modulate its immunity against *P. carotovorum* subsp. *carotovorum*. Recent observations in our laboratory indicated that bacterial virulence and disease severity caused by *P. carotovorum* subsp. *carotovorum* were reduced by physically separate application of *B. subtilis* GB03, which emits 2,3-butanediol as the major BVC (data not shown). Further investigations detected other BVCs which are 2,3-butanediol and 2,3-butanedione from *B. subtilis*, which negatively regulated the post-transcriptional regulator of virulence factors, RsmA, in *P. carotovorum* subsp. *carotovorum* (Kõiv et al. 2013; Broberg et al. 2014; unpublished data, Lee and Ryu). Inactivation of RsmA suppressed transcription of virulence factors but did not affect bacterial multiplication in the plant.

We still do not fully understand how plants differentially perceive the 2,3-butanediol produced by beneficial and pathogenic bacteria. It is difficult to speculate that the plants can discriminate from which group of bacteria one same chemical compound originates. We hypothesize that the key factor in this differentiation is timing similarly to the "first come, first served" principle. Under natural conditions, *B. subtilis* colonizes the plant surface and emits sufficient BVCs to elicit induced plant resistance against a broad range of pathogens. When *P. carotovorum* colonizes the plant first, the virulence factors initially break through plant resistance and cause disease before the plant establishes full resistance. This hypothesis is consistent with many studies reporting a lag time of 3–4 days between application of beneficial bacteria and elicitation of plant induced resistance (Ryu et al. 2004a, b). Intriguingly, we recently identified that certain BVCs from *B. subtilis* also suppress virulence factor transcription in *P. carotovorum*, thereby suppressing pathogenicity indicating that more direct chemical interactions can occurr in nature (unpublished data, Lee and Ryu).

## Metabolic engineering of PGPR to improve and expand BVC production

Bacterial metabolic engineering has been utilized to improve or expand BVC production. We review recent studies conducting metabolic engineering of 2,3-butanediol and acetoin in model bacteria and several PGPR species.

Recent studies performing metabolic engineering of BVCs have aimed to improve biofuel production (Peralta-Yahya and Keasling 2010). The C4 alcohol–butanol has an energy density of 29 MJ/L, which is similar to that of gasoline (32 MJ/L) (Atsumi et al. 2008). Many C4 biofuels are highly toxic to bacteria; however, 2,3-butanediol has low toxicity and stable chemical properties (e.g., 27,200 J/g heating value) (Zeng and Sabra 2011; Oliver et al. 2013). Many species of *Klebsiella*, *Enterobacter*, *Serratia*, *Paenibacillus*, and *Bacillus* are natural 2,3-butanediol producers (Li et al. 2013). Microbial production of 2,3-butanediol has been extensively reviewed elsewhere (Garg and Jain 1995; Celinska and Grajek 2009; Ji et al. 2010; Nielsen et al. 2010; Ji et al. 2011) and will not be discussed in this review.

### Engineering 2,3-butanediol production in a model *E. coli*

The initial studies selected *E. coli* because of the lack of a functional 2,3-butanediol biosynthesis pathway (Ui et al. 1997). *E. coli* is a 2,3-butanediol-negative species according to the Voges–Proskauer test, which is the classic test of bacterial production of 2,3-butanediol (Xu et al. 2014). The experimental aim was to boost 2,3-butanediol production in the engineered *E. coli*. The general strategy used for these experiments could be useful for engineering PGPR species (Xu et al. 2014) (Fig. 2).

Three enzymes are required for 2,3-butanediol biosynthesis: α-acetolactate synthase (ALS; EC 4.1.3.18), α-acetolactate decarboxylase (ALDC; EC 4.1.1.5), and 2,3-butanediol dehydrogenase (BDH; EC 1.1.1.76; also called acetoin reductase, EC 1.1.1.4) (Celinska and

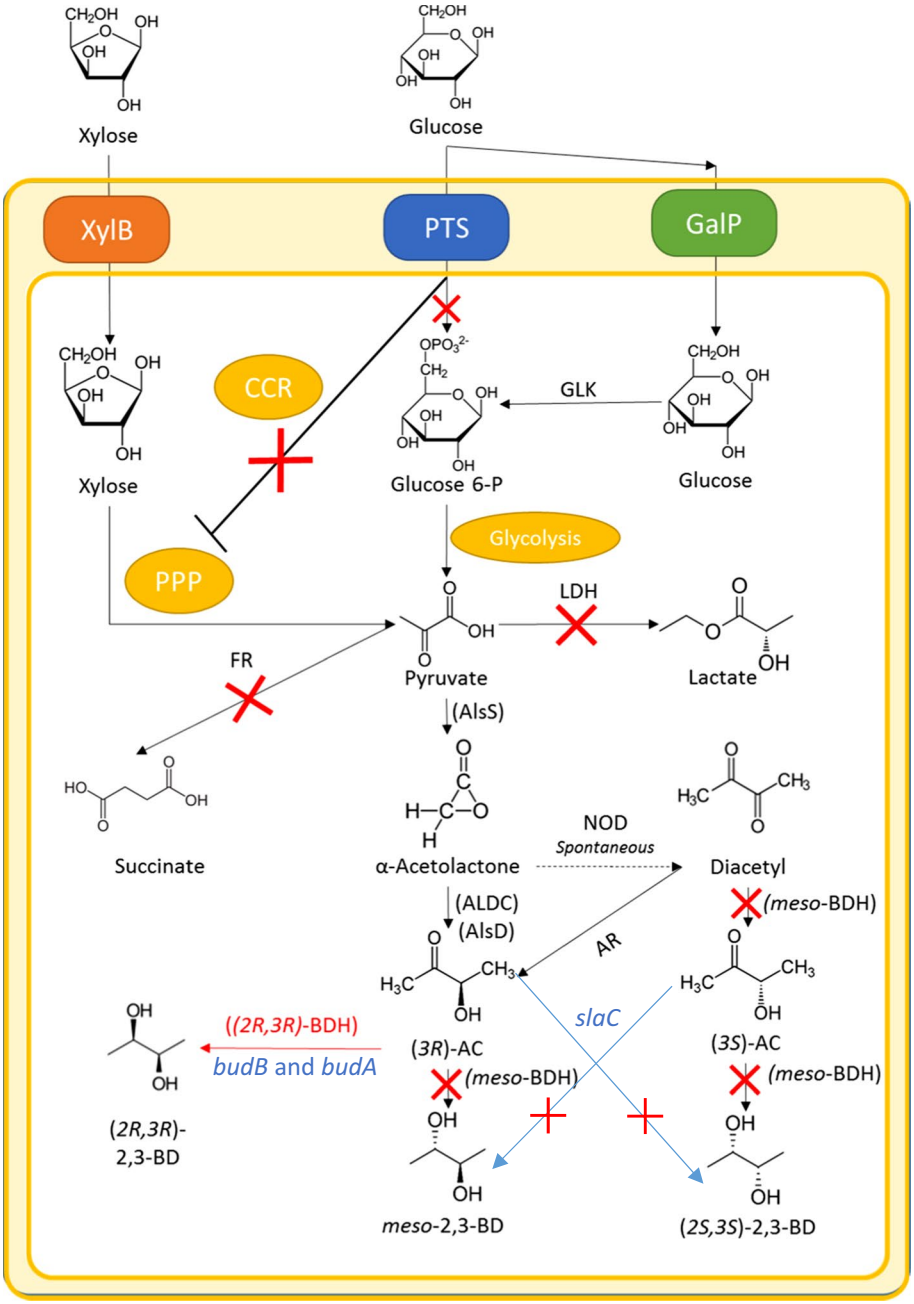


**Fig. 2** Representative metabolic engineering strategy to increase production of (*2R,3R*)-2,3-butanediol in Gram-negative bacteria (*ALDC* α-acetolactate decarboxylase, *ALS* α-acetolactate synthase, *AR* acetoin reductase, *BDH* 2,3-butanediol dehydrogenase, *CCR* carbon catabolite repression, *FR* fumarate reductase, *GalP* galactose permease, *GLK* glucose kinase, *LDH* lactate dehydrogenase, *NOD* non-enzymatic oxidative decarboxylation, *PPP* pentose phosphate pathway, *PTS* phosphotransferase system, *XylB* D-xylose transporter). *Red crosses* indicate deleted genes; *red arrow* indicates engineered genes; *Blue genes* and *arrows* remarked exogenous components for metabolite engineering. The pathway was modified from previous publications (Li et al. 2013; Li et al. 2014; Xu et al. 2014)

Grajek 2009; Ji et al. 2011; Zhang et al. 2013). ALS catalyzes the condension of two pyruvate molecules to yield α-acetolactate (Celinska and Grajek 2009; Ji et al. 2011). Then, ALDC converts α-acetolactate to (*3R*)-acetoin (Celinska and Grajek 2009; Ji et al. 2011). Finally, BDH reduces (*3R*)-acetoin to meso-2,3-butanediol, and meso-2,3-butanediol is converted to (*2R,3R*)-2,3-butanediol (Celinska and Grajek 2009; Ji et al. 2011; Zhang et al. 2013). The 2,3-butanediol biosynthetic gene cluster in *B. subtilis* 168 has been cloned and characterized (Renna et al. 1993). The major obstacle to produce an active 2,3-butanediol [(*2R,3R*)-butanediol] is to selectively modify bacterial metabolic pathways not to produce the stereoisomers [(*2S,3S*)-butanediol and meso-2,3-butanediol]. A recent study reported the production of enatiomerically pure (*2R,3R*)-2,3-butanediol in *E. coli* by engineering the expression of *budB* and *budA* from *Klebsiella pneumoniae* and *ydjL* from *B. subtilis*. The three genes were constructed as a plasmid expression vector, pTrc99a-*budB*-*budA*-*ydjL*, which was regulated by a *Trc* promoter in *E. coli* MG1655 (Ji et al. 2014). The YdjL enzyme (BDH) specifically targets and converts the R-form of acetoin into (*2R,3R*)-butanediol.

### Engineering plant-associated bacteria to boost production of 2,3-butanediol

Certain Gram-negative and -positive bacteria and PGPR species produce 2,3-butenadiol but do not reach the functional levels required for beneficial action in the plants. The goal of the studies is to improve 2,3-butanediol production in species with low levels by introducing exogenous genes for overexpression studies. We discuss the results of studies conducting metabolic engineering on *Bacillus*, *Serratia*, and *Enterobacter*.

*B. amyloliquefaciens* is well-characterized as an inducer of plant growth and immunity (Rudrappa et al. 2010) via production of 2,3-butanediol. Researchers overexpressed a *GapA* gene encoding $NAD^+$-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which oxidizes glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate and results in high pyruvate levels, the major precursor for 2,3-butanediol production (Yang et al. 2013). Overexpression of *gapA* in *B. amyloliquefaciens* increased 2,3-butanediol production without inhibiting cell growth. However, the glucose consumption rate was lower than that of wild type, possibly due to higher NADH pools and lower $NAD^+$ pools, which would increase the [NADH]/[$NAD^+$] ratio. To solve this problem, the gene encoding 2,3-butanediol dehydrogenase, which catalyzes the conversion of acetoin to 2,3-butanediol from *B. amyloliquefaciens* was cotransformed with *GapA*. Co-expression of *GapA* and *Bdh* in *B. amyloliquefaciens* increased 2,3-butanediol levels by 26 % and reduced acetoin levels by 83 % compared with controls (Yang et al. 2013). However, the engineered *B. amyloliquefaciens* did not produce the active 2,3-butanediol stereoisomer [(*2R,3R*)-2,3-butanediol].

A *SlaC* gene was identified in *S. marcescens* (Bai et al. 2015), which encoded a meso-2,3-butanediol dehydrogenase that converts (*3R*)-acetoin and (*3S*)-acetoin to meso-2,3-butanediol and (*2S,3S*)-2,3-butanediol, respectively. Inactivation of *SlaC* (to generate the *slaC* mutant) and overexpression of *Bdh* (from *B. subtilis*) in *S. marcescens* might produce a high level of pure (*2R,3R*)-2,3-butanediol, but this remains to be tested. A similar approach was used for metabolic engineering of *Enterobacter cloacae* (Li et al. 2015).

A systematic approach was used to engineer multiple target genes in *E. cloacae*. This study implemented the following engineering strategy: (1) biosynthesis of undesired 2,3-butanedione stereoisomers was blocked by disrupting a meso-2,3-butanediol dehydrogenase; (2) genes related to the two-carbon catabolite-repression genes *PtsG* and *GalP* were deleted, which induced utilization of the lignocellulose-derived sugars glucose and xylose; and (3) genes responsible for enzymes that catalyze the production of 2,3-butanediol derivatives, *Ldh* and *FrdA*, were deleted to promote the production of the desired stereoisomer. These three modifications of *E. cloacae* metabolic pathways resulted in a 16 % increase in (*2R,3R*)-2,3-butanediol production (with >97 % purity) compared with that of the wild type (Li et al. 2015).

## Unresolved questions regarding the biological relevance of BVCs

The current body of evidence for BVCs has not completely resolved questions regarding their biological relevance under natural conditions. To study the effects of BVCs, researchers primarily used in vitro systems such as the I-plate (a Petri dish containing two compartments separated by a barrier), the Y-plate (a Petri dish containing three compartments separated by barriers), the X-plate (a Petri dish containing four compartments), and multi-well microtiter plates (Ryu et al. 2003; Blom et al. 2011; Lee et al. 2012). The effects observed using these in vitro systems may differ in natural environments such as the rhizosphere. In this section, we summarize the problematic issues and present recent advances toward testing BVC functions in natural environments.

### Is the BVC effect a side effect of $CO_2$ on plant growth?

The most critical argument against a positive effect of BVCs on plant growth and immunity involves the effect of $CO_2$ (Kai and Piechulla 2009). *Serratia odorifera* was reported previously as a PGPR strain through the bacteria emitted several volatile compounds (Kai and Piechulla 2009) including $CO_2$, which sharply increased from 390 to 3000 ppm after 24 h of plant treatment. The main reason for this large $CO_2$ accumulation was because *S. odorifera* was grown in sealed Petri dishes. Treatment of plants with *S. odorifera* in open-system conditions (without sealing the Petri dishes) and treatment of plants with 0.1 M $Ba(OH)_2$ (captured as atmospheric $CO_2$) abolished the positive effects of *S. odorifera* on plant growth (Kai and Piechulla 2009). $CO_2$ emission in the bacterial rhizosphere has been shown to modulate plant growth; however, recent studies suggest that the effect of bacterial $CO_2$ on plant growth promotion may be only marginally significant.

A BVC of the PGPR *Paenibacillus polymyxa* E681 isolated from winter barley elicited a 60 % increase in the total leaf surface area of *Arabidopsis* compared with water-treated control plants (Lee et al. 2012). Additional experiments showed that *P. polymyxa* E681 also promoted *Arabidopsis* growth when $CO_2$ was captured with 0.1 M $Ba(OH)_2$, suggesting that $CO_2$ may not be the only bacterial factor promoting growth (Lee et al. 2012). Additionally, In vitro application of chemically BVCs except for

**Table 1** Case studies of BVC soil application under greenhouse or field conditions

| BVCs | Plants | Function | Target pathogens or insects | Application condition | References |
|---|---|---|---|---|---|
| 2,3-butanediol acetoin | *Arabidopsis thaliana* | Growth promotion | *Pectobacterium carotovorum* subsp. *carotovorum* | Greenhouse | Ryu et al. (2005a, b) |
| 2,3-butanediol | *Nicotiana benthamiana* | – | *Colletotrichum orbiculare* | Greenhouse | Cortes-Barco et al. (2010a) |
| 2,3-butanediol | *Capsicum annuum* | Growth promotion | – | Greenhouse | Hahm et al. (2012) |
| 2,3-butanediol 2-butanol | *Zea mays* | Recruiting wasp *Cotesia marginiventris* | *Spodoptera littoralis* | Field | D'Alessandro et al. (2014) |
| Dimethyl disulfide | *N. tabacum* and *Zea mays* | Growth promotion | *Botrytis cinerea* and *Cochliobolus heterostrophus* | Greenhouse | Huang et al. (2012) |
| 2-butanone | *Cucumis sativus* | Recruiting ladybird *Coccinella magnifica* | *Pseudomonas syringae* pv. lachrymans and *Myzus persicae* | Field | Song and Ryu (2013) |

$CO_2$ effects was sufficient to promote plant growth and immunity in *Arabidopsis* (Kai and Piechulla 2009). Single chemical BVCs application showed growth promotion and eliciting ISR in previous studies.

## Are BVCs analyzed primarily in artificial and closed systems?

Researchers investigating volatile compounds generally capture the compounds by sealing the experimental setup, which is an artificial system. Even experimental setups feeding a continuous flow of volatile compounds do not realistically model natural systems. Closed systems result in continual accumulation of specific BVCs on artificial medium until nutrients are depleted. After researchers obtained evidence for BVC-mediated stimulation of plant growth or immunity, most studies profiled and identified the active BVCs, which were then verified by pharmaceutical application on the test plants. However, the concentrations of isolated BVCs were much higher than the concentrations in bacterial emissions in nature (Blom et al. 2011; Lee et al. 2012).

An examination of the role of BVCs under natural conditions was reported recently. Quantitative solid-phase microextraction (SPME)-GC/MS analysis indicated that 2,3-butanediol and acetoin were emitted from maize leaves, sheath, and seeds (D'Alessandro et al. 2014). Seeds were surface-sterilized, and *Enterobacter aerogenes* (syn. *Klebsiella mobilis*) was isolated only from seeds emitting 2,3-butanediol, indicating the presence of an endophytic bacterium. Further analysis confirmed that the bacteria produced a large amount of 2,3-butanediol. The emitting maize seeds were planted, and the resulting plants were analyzed (D'Alessandro et al. 2014). The bacteria were observed colonizing the interior of both above-ground and below-ground maize tissues. These results suggest that plant-associated bacteria can colonize internal plant tissues under natural conditions and release detectable BVCs, which affect plant physiology and promote plant growth and induce defense (D'Alessandro et al. 2014).

## Does application of BVCs into soil promote plant growth and immunity?

To utilize BVCs in agricultural crops, the volatile compounds have to be applied under open-field conditions, which are very different from the in vitro conditions used during experimental studies. The effectiveness of BVCs in such large, open systems has to be proven because of dilution and evaporation effects. There are few studies assessing the effects of BVC application under open conditions. However, soil applications of BVCs have been reported, and some potentially promising results were obtained. This section discusses the available studies of BVC application into soil and its effects on plant growth and resistance to fungal, bacterial, viral, and insect herbivore pathogens depending on year (Table 1).

Since it was discovered that *Bacillus* spp. 2,3-butanediol elicited plant growth and ISR (Ryu et al. 2003, 2004a, b), many studies applied 2,3-butanediol into soil of open fields to test its effects under agricultural conditions. The first trial that applied 2,3-butanediol and acetoin into soil resulted in an increase in leaf fresh weight of *Arabidopsis* compared with water-treated controls (Ryu et al. 2005a, b). The (*2R,3R*)-butanediol isomer had greater activity in eliciting ISR in *Arabidopsis* and tobacco than (*2S,3S*)-butanediol (Ryu et al. 2004a, b; Han et al. 2006). Later, a 10 mL drench application of 100 μM (*2R,3R*)-butanediol into soil activated ISR against the anthracnose fungus *Colletotrichum orbiculare* on *Nicotiana benthamiana*

leaves (Cortes-Barco et al. 2010a). Transcriptional evaluation of six *Pathogenesis-related* (*PR*) genes showed that transcript levels of *NbPRb-1b*, *NbPR-2*, and *NbPR-5 dB* were significantly elevated only after the (*2R*,*3R*)-butanediol drench application and subsequent challenge with *C. orbiculare*, but not after drench application of water or of (*2R,3R*)-butanediol alone, without subsequent challenge with the pathogen. These results indicate that (*2R*,*3R*)-butanediol elicits defense priming of the three *PR* genes. The JA/ET signaling pathway is known to modulate ISR (Xu et al. 1994; Okubara and Paulitz 2005), and defense priming is one of the primary mechanisms mediating ISR (Ton et al. 2007). The drench application study reported the first molecular genetic evidence for BVC-induced ISR. Similarly drench applications of 2,3-butanediol and acetoin into soil under greenhouse conditions resulted in 15.2 and 12.4 % higher fresh weight of pepper, respectively, than those of control plants (Hahm et al. 2012).

The ketone BVC 2-butanone emitted from *B. amyloliquefaciens* IN937a and *B. subtilis* GB03 triggered ISR in cucumber against a bacterial pathogen and an insect herbivore (Song and Ryu 2013). Pretreatment of cucumber seedlings with 10 nM and 0.1 μM 2-butanone stimulated protection against angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans* in large-scale trials (Song and Ryu 2013), and total fruit weight was greater for cucumber plants treated with 0.1 μM 2-butanone. The numbers of aphid (*Myzus persicae*) nymphs and adults were significantly less on cucumber plants pretreated with 10 nM and 0.1 μM 2-butanone than on control plants, whereas the number of ladybirds, a natural enemy of aphids, was significantly greater (Song and Ryu 2013). *CsLOX* transcripts on application of 2-butanone were up-regulated, which indicates induction of oxylipin biosynthetic pathways. These results indicate that tritrophic interactions (indirect defense) were induced in cucumber by the emission of green leaf volatiles, which recruit natural enemies of plant insect pests (Mercke et al. 2004; Kappers et al. 2010). These studies were the first reports of BVC-mediated recruitment of a natural enemy of an attacking insect.

Screens for ISR-inducing BVCs from *B. cereus* C1L identified dimethyl disulfide (Huang et al. 2012). The effectiveness of dimethyl disulfide was examined by drenching the soil of tobacco and maize with 0.1, 1, and 10 mM dimethyl disulfide under greenhouse conditions. Treatments with 1 and 10 mM dimethyl disulfide augmented systemic resistance in tobacco and maize against *B. cinerea* and *C. heterostrophus*, respectively (Huang et al. 2012). Direct evidence for the involvement of BVCs as triggers of plant protection was reported in a study of interactions between the endophytic bacterium *Enterobacter aerogenes* and maize (D'Alessandro et al. 2014). Soil application of 2 mg/mL 2,3-butanediol to maize seedlings caused a three-fold reduction in the necrotic area caused by Northern corn leaf blight (*Setosphaeria turcica*), whereas application of the structurally similar compound 2-butanol had no significant affect. Olfactometer data evaluating the preference of the wasp *Cotesia marginiventris* that were herbivore parasitic insect against *Spodoptera littoralis* caterpillars showed that corn plants treated with a soil drench of 2,3-butanediol became more attractive, whereas the 2,3-butanediol soil drench did not affect the preference of caterpillars. Treatment with 2,3-butanediol had no negative effects on fungal and insect growth.

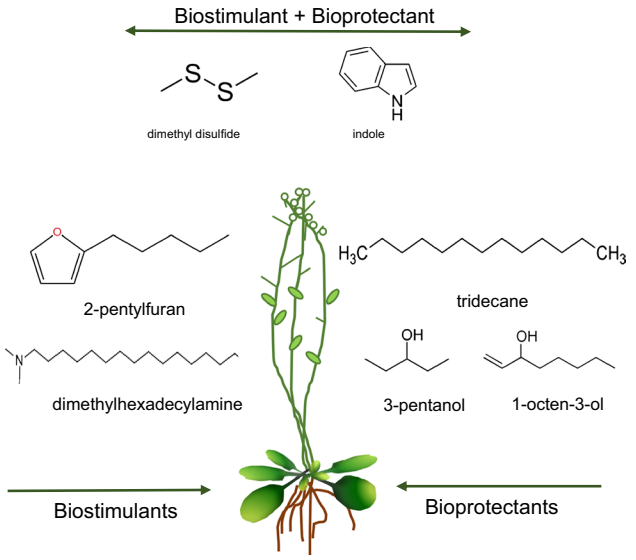


**Fig. 3** Recently identified plant biostimulant and bioprotectant volatile compounds emitted from bacteria. Indole and dimethyl disulfide function as biostimulants and bioprotectants; 2-pentylfuran and dimethylhexadecylamine function as biostimulants (Zou et al. 2010; Velázquez-Becerra et al. 2010; Meldau et al. 2013; Bailly et al. 2014); tridecane, 3-petanol, and 1-octen-3-ol function as bioprotectants (Gutiérrez-Luna et al. 2010; Lee et al. 2012; Huang et al. 2012; Choi et al. 2014; Erb et al. 2015)

## New kids on the block

This section introduces new BVCs that were recently shown to enhance plant growth and immunity. We classify BVCs into two functional groups as biostimulants or bioprotectants (Fig. 3).

### BVCs as biostimulants

#### *Indole*

Indole is a BVC isolated from soil-borne bacteria. Indole promotes *Arabidopsis* growth and has a crucial role in lateral

root growth, which can be mediated by disrupting the auxin-signaling machinery (Bailly et al. 2014). Recent work used the auxin reporter *DR5*::GUS lines and classic auxin physiological and transport assays to show that plants can convert bacterial indole to auxin (indole acetic acid), and the polar auxin transport machinery is crucial for indole-induced lateral root growth. These results indicate that bacterial indole can function as a messenger regulating plant growth and development (Lee and Lee 2010; Bailly et al. 2014).

*Dimethyl disulfide*

The exact mechanism mediating BVC-induced plant growth has not been completely elucidated (Bailly and Weisskopf 2012). A study of the interaction between *Nicotiana attenuata* and its naturally root-associated *Bacillus* sp. B55 identified the sulfur (S)-containing BVC dimethyl disulfide (DMDS), which was determined to have a crucial role in enhancing plant growth by increasing plant sulfur content (Meldau et al. 2013). This mechanism was confirmed in *ethylene-insensitive 35S-ethylene response 1* (*35S-etr1*) mutants, which are impaired in $SO_4^{2-}$ uptake and S metabolism. Treatment of *35S-etr1* plants with DMDS and BVCs from *Bacillus* sp. B55 rescued the mutant plant phenotypes (lack of root hairs, poor lateral root growth, and low chlorophyll content) (Meldau et al. 2013).

*2-Pentylfuran*

Zou et al. (2010) reported that the fresh weight of *Arabidopsis* increased approximately two-fold after exposure to *Bacillus megaterium* XTBG34 BVCs compared with that of the control, which was treated with *E. coli* DH5α BVCs. Eleven BVCs were identified in strain XTBG34, including aldehyde, alkanes, ketones, and aroma compounds. The BVC 2-pentylfuran was identified as a key volatile promoting plant growth (Zou et al. 2010). An early study reported 2-pentylfuran emission from *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a; however, 2-pentylfuran was not studied earlier because of its trace amounts in bacterial emissions (Farag et al. 2006).

*Dimethylhexadecylamine*

BVCs of *Arthrobacter agilis* UMCV2, a PGPR strain isolated from maize, promoted the growth of *Medicago sativa* seedlings as measured by increases in root length, stem length, and plant biomass (Velázquez-Becerra et al. 2010). The volatile compounds *N,N*-dimethylhexadecylamine (dimethylhexadecylamine) and lipoamino acids structurally related to bacterial QS signals were identified as key compounds mediating enhanced plant growth.

## BVCs as bioprotectants

*Indole*

Indole is a ubiquitous chemical produced in many Gram-positive and Gram-negative bacteria. Indole has multiple roles in spore formation, signaling, plasmid stability, drug resistance, biofilm formation, and virulence (Lee and Lee 2010). Indole is a BVC that activates root development by interfering with auxin signaling mediated by plant-bacterial inter-kingdom communication. Direct application of indole on maize plants primes defense responses via jasmonic acid signaling (Bailly et al. 2014; Erb et al. 2015).

*Dimethyl disulfide*

The PGPR *Bacillus cereus* C1L elicits ISR and protects plants from the necrotrophic fungal pathogen *B. cinerea*. The *B. cereus* C1L ISR elicitor was identified by SPME-GC/MS as dimethyl disulfide, which is a volatile compound that protects tobacco and maize against *B. cinerea* and *Cochliobolus heterostrophus* in tobacco and maize. Dimethyl disulfide function showed that it could be a potential ISR elicitor in *B. cereus* C1L (Huang et al. 2012).

*Tridecane*

The PGPR *Paenibacillus polymyxa* E681 was isolated from winter barley roots; it promotes plant biomass production and functions to control phytopathogens (Lee et al. 2012). Tridecane is a $C_{13}$ alkane hydrocarbon emitted by E681. Tridecane induces systemic resistance in *Arabidopsis* against *Pseudomonas syringae* pv. *maculicola* ES4326 by priming gene expression within salicylic acid (SA), JA, and ethylene (ET) signaling pathways.

*Pentanol and its derivatives*

An initial study reported that the PGPR *Bacillus amyloliquefaciens* strain IN937a induced plant systemic resistance via BVC emissions (Ryu et al. 2004a, b). Pepper resistance induced by BVCs and their derivatives emitted by strain IN937a reduced bacterial spot disease caused by *Xanthomonas axonopodis* pv. *vesicatoria*. A search for the key BVCs identified 3-pentanol out of 15 candidate BVCs of IN937a (Choi et al. 2014). Previous studies showed that 3-pentanol was a volatile emitted by plants and insects, but it was rarely detected in bacteria. The production of 1-pentanol was detected in bacteria (*Bacillus* spp.) isolated from soil; 1-pentanol strongly reduced phytopathogen infection in pepper (data not shown). The derivative 3-pentanol alleviated the pepper disease severity caused by *X. axonopodis* and naturally occurring *Cucumber mosaic virus*, and

RT-PCR analysis showed that pentanol elevated the expression of defense-related genes involved in SA, JA, and ET signaling pathways (including *Pathogenesis-related* genes) (Choi et al. 2014). Therefore, pentanol and its derivatives have potential as candidate bioprotectants.

*1-Octen-3-ol*

1-Octen-3-ol is a major and widespread volatile compound produced by mushrooms (Börjesson et al. 1992). Vaporized 1-octen-3-ol emitted by mold fungi enhances JA/ET-dependent and wounding-dependent plant gene expression, and reduces disease symptoms caused by *Botrytis cinerea*, a necrotrophic fungal pathogen of *Arabidopsis* (Kishimoto et al. 2007). Other case study reported that vaporized 1-octen-3-ol reduced germination of *Lecanicillium fungicola*, which causes dry bubble disease (Berendsen et al. 2013). Especially, the bacterial volatile 1-octen-3-ol was reported in the rhizospheric bacterial strain L255 presumed to be a PGPR strains. These results suggest that the BVC 1-octen-3-ol is a promising bioprotectant (Gutiérrez-Luna et al. 2010).

## Conclusions and perspectives

The improvement of plant growth and immunity by BVCs has been established. This review summarized recent advances in BVC-mediated plant growth and immunity. Several recently identified BVCs require further characterization and functional analyses. Greater progress is required to move the experiments from in vitro closed conditions to open-field studies with relevance for large-scale agricultural applications. New strategies are required to resolve long-standing technical problems including rapid evaporation of volatile compounds and inconsistent experimental effects. Metabolic engineering of beneficial strains to improve and/or expand BVC production holds great promise for future applications. Several technical challenges must be resolved, which will require the input of synthetic biology, genomics, and bioengineering. Metabolic engineering has agricultural and industrial applications, for greater plant productivity and for biofuel production, respectively. Future studies should examine the basic ecology of BVCs in natural systems and its effects on plants, insects, and nematodes. Compared with the potential scope and impact of BVC applications in the future, the field can be considered to still be in its infancy.

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## References

Atsumi S et al (2008) Metabolic engineering of *Escherichia coli* for 1-butanol production. Metab Eng 10:305–311. doi:10.1016/j.ymben.2007.08.003

Audrain B, Farag MA, Ryu CM, Ghigo JM (2015) Role of bacterial volatile compounds in bacterial biology. FEMS Microbiol Rev 39:222–233. doi:10.1093/femsre/fuu013

Bai F, Dai L, Fan J, Truong N, Rao B, Zhang L, Shen Y (2015) Engineered *Serratia marcescens* for efficient (*3R*)-acetoin and (*2R,3R*)-2,3-butanediol production. J Ind Microbiol Biotechnol 42:779–786. doi:10.1007/s10295-015-1598-5

Bailly A, Weisskopf L (2012) The modulating effect of bacterial volatiles on plant growth: current knowledge and future challenges. Plant Signal Behav 7:79–85. doi:10.4161/psb.7.1.18418

Bailly A, Groenhagen U, Schulz S, Geisler M, Eberl L, Weisskopf L (2014) The inter-kingdom volatile signal indole promotes root development by interfering with auxin signalling. Plant J 80:758–771. doi:10.1111/tpj.12666

Bengtsson JM et al (2009) Field attractants for *Pachnoda interrupta* selected by means of GC-EAD and single sensillum screening. J Chem Ecol 35:1063–1076. doi:10.1007/s10886-009-9684-7

Berendsen R, Kalkhove SC, Lugones L, Baars JP, Wösten HB, Bakker PHM (2013) Effects of the mushroom-volatile 1-octen-3-ol on dry bubble disease. Appl Microbiol Biotechnol 97:5535–5543. doi:10.1007/s00253-013-4793-1

Bernier SP, Letoffe S, Delepierre M, Ghigo JM (2011) Biogenic ammonia modifies antibiotic resistance at a distance in physically separated bacteria. Mol Microbiol 81:705–716. doi:10.1111/j.1365-2958.2011.07724.x

Bitas V, Kim HS, Bennett JW, Kang S (2013) Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health. Mol Plant Microbe Interact 26:835–843. doi:10.1094/MPMI-10-12-0249-CR

Blom D, Fabbri C, Eberl L, Weisskopf L (2011) Volatile-mediated killing of *Arabidopsis thaliana* by bacteria is mainly due to hydrogen cyanide. Appl Environ Microbiol 77:1000–1008. doi:10.1128/AEM.01968-10

Börjesson T, Stöllman U, Schnürer J (1992) Volatile metabolites produced by six fungal species compared with other indicators of fungal growth on cereal grains. Appl Environ Microbiol 58:2599–2605

Broberg M, Lee GW, Nykyri J, Lee YH, Pirhonen M, Palva ET (2014) The global response regulator ExpA controls virulence gene expression through RsmA-mediated and RsmA-independent pathways in *Pectobacterium wasabiae* SCC3193. Appl Environ Microbiol 80:1972–1984. doi:10.1128/AEM.03829-13

Buttery RGKJ, Ling LC (1984) Volatile components of red clover leaves, flowers, and seed pods: possible insect attractants. J Agric Food Chem 32:254–256

Celinska E, Grajek W (2009) Biotechnological production of 2,3-butanediol–current state and prospects. Biotechnol Adv 27:715–725. doi:10.1016/j.biotechadv.2009.05.002

Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, Lee YH, Cho BH, Yang KY, Ryu CM, Kim YC (2008) 2R,3R-butanediol, a bacterial volatile produced by Pseudomonas chlororaphis O6, is involved in induction of systemic tolerance to drought in Arabidopsis thaliana. Mol Plant Microbe Interact 21(8):1067–1075. doi: 10.1094/MPMI-21-8-1067

Choi HK, Song GC, Yi HS, Ryu CM (2014) Field evaluation of the bacterial volatile derivative 3-pentanol in priming for induced resistance in pepper. J Chem Ecol 40:882–892. doi:10.1007/s10886-014-0488-z

Cortes-Barco AM, Hsiang T, Goodwin PH (2010a) Induced systemic resistance against three foliar diseases of *Agrostis stolonifera* by (*2R,3R*)-butanediol or an isoparaffin mixture. Ann Appl Biol 157:179–189

Cortes-Barco AM, Goodwin PH, Hsiang T (2010b) Comparison of induced resistance activated by benzothiadiazole, (*2R,3R*)-butanediol and an isoparaffin mixture against anthracnose of *Nicotiana benthamiana*. Plant Pathol 59:643–653

D'Alessandro M, Erb M, Ton J, Brandenburg A, Karlen D, Zopfi J, Turlings TC (2014) Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. Plant, Cell Environ 37:813–826. doi:10.1111/pce.12220

Effantin G, Rivasseau C, Gromova M, Bligny R, Hugouvieux-Cotte-Pattat N (2011) Massive production of butanediol during plant infection by phytopathogenic bacteria of the genera *Dickeya* and *Pectobacterium*. Mol Microbiol 82:988–997. doi:10.1111/j.1365-2958.2011.07881.x

Effmert U, Kalderas J, Warnke R, Piechulla B (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38:665–703. doi:10.1007/s10886-012-0135-5

Erb M, Veyrat N, Robert CAM, Xu H, Frey M, Ton J, Turlings TCJ (2015) Indole is an essential herbivore-induced volatile priming signal in maize. Nat Commun 6:6273. doi:10.1038/ncomms7273

Farag MA, Ryu CM, Sumner LW, Pare PW (2006) GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. Phytochemistry 67:2262–2268. doi:10.1016/j.phytochem.2006.07.021

Farag MA, Zhang H, Ryu CM (2013) Dynamic chemical communication between plants and bacteria through airborne signals: induced resistance by bacterial volatiles. J Chem Ecol 39:1007–1018. doi:10.1007/s10886-013-0317-9

Garg SK, Jain A (1995) Fermentative production of 2,3-butanediol: a review. Bioresour Technol 51:103–109. doi:10.1016/0960-8524(94)00136-O

Gutiérrez-Luna F, López-Bucio J, Altamirano-Hernández J, Valencia-Cantero E, de la Cruz H, Macías-Rodríguez L (2010) Plant growth-promoting rhizobacteria modulate root-system architecture in *Arabidopsis thaliana* through volatile organic compound emission. Symbiosis 51:75–83. doi:10.1007/s13199-010-0066-2

Hahm MS et al (2012) Biological control and plant growth promoting capacity of rhizobacteria on pepper under greenhouse and field conditions. J Microbiol 50:380–385. doi:10.1007/s12275-012-1477-y

Han SH, Lee SJ, Moon JH, Park KH, Yang KY, Cho BH, Kim KY, Kim YW, Lee MC, Anderson AJ, Kim YC (2006) GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco. Mol Plant Microbe Interact 19:924–930. doi:10.1094/mpmi-19-0924

Hsieh SC, Lu CC, Horng YT, Soo PC, Chang YL, Tsai YH, Lin CS, Lai HC (2007) The bacterial metabolite 2,3-butanediol ameliorates endotoxin-induced acute lung injury in rats. Microbes Infect 9:1402–1409. doi:10.1016/j.micinf.2007.07.004

Huang CJ, Tsay JF, Chang SY, Yang HP, Wu WS, Chen CY (2012) Dimethyl disulfide is an induced systemic resistance elicitor produced by *Bacillus cereus* C1L. Pest Manag Sci 68:1306–1310. doi:10.1002/ps.3301

Ji XJ, Huang H, Zhu JG, Ren LJ, Nie ZK, Du J, Li S (2010) Engineering *Klebsiella oxytoca* for efficient 2,3-butanediol production through insertional inactivation of acetaldehyde dehydrogenase gene. Appl Microbiol Biotechnol 85:1751–1758. doi:10.1007/s00253-009-2222-2

Ji XJ, Nie ZK, Huang H, Ren LJ, Peng C, Ouyang PK (2011) Elimination of carbon catabolite repression in *Klebsiella oxytoca* for efficient 2,3-butanediol production from glucose-xylose mixtures. Appl Microbiol Biotechnol 89:1119–1125. doi:10.1007/s00253-010-2940-5

Ji XJ, Liu LG, Shen MQ, Nie ZK, Tong YJ, Huang H (2014) Constructing a synthetic metabolic pathway in Escherichia coli to produce the enantiomerically pure (R,R)-2,3-butanediol. Biotechnol Bioeng 112:1056–1059. doi:10.1002/bit.25512

Kai M, Piechulla B (2009) Plant growth promotion due to rhizobacterial volatiles—An effect of $CO_2$? FEBS Lett 583:3473–3477. doi:10.1016/j.febslet.2009.09.053

Kanchiswamy CN, Malnoy M, Maffei ME (2015) Bioprospecting bacterial and fungal volatiles for sustainable agriculture. Trends Plant Sci 20:206–211. doi:10.1016/j.tplants.2015.01.004

Kappers IF, Verstappen FW, Luckerhoff LL, Bouwmeester HJ, Dicke M (2010) Genetic variation in jasmonic acid- and spider mite-induced plant volatile emission of cucumber accessions and attraction of the predator *Phytoseiulus persimilis*. J Chem Ecol 36:500–512. doi:10.1007/s10886-010-9782-6

Kishimoto K, Matsui K, Ozawa R, Takabayashi J (2007) Volatile 1-octen-3-ol induces a defensive response in *Arabidopsis thaliana*. J Gen Plant Pathol 73:35–37. doi:10.1007/s10327-006-0314-8

Kõiv V et al (2013) Lack of RsmA-mediated control results in constant hypervirulence, cell elongation, and hyperflagellation in *Pectobacterium wasabiae*. PLoS ONE 8:e54248. doi:10.1371/journal.pone.0054248

Kwan G, Charkowski AO, Barak JD (2013) *Salmonella enterica* suppresses *Pectobacterium carotovorum* subsp. *carotovorum* population and soft rot progression by acidifying the microaerophilic environment. MBio 4:e00512–e00557. doi:10.1128/mBio.00557-12

Lee JH, Lee J (2010) Indole as an intercellular signal in microbial communities. FEMS Microbiol Rev 34:426–444. doi:10.1111/j.1574-6976.2009.00204.x

Lee B, Farag MA, Park HB, Kloepper JW, Lee SH, Ryu CM (2012) Induced resistance by a long-chain bacterial volatile: elicitation of plant systemic defense by a C13 volatile produced by *Paenibacillus polymyxa*. PLoS ONE 7:e48744. doi:10.1371/journal.pone.0048744

Li J, Wang W, Ma Y, Zeng AP (2013) Medium optimization and proteome analysis of (R,R)-2,3-butanediol production by *Paenibacillus polymyxa* ATCC 12321. Appl Microbiol Biotechnol 97:585–597. doi:10.1007/s00253-012-4331-6

Li L, Wang Y, Li K, Su F, Ma C, Xu P (2014) Genome sequence of meso-2,3-butanediol-producing strain *Serratia marcescens* ATCC 14041. Genome Announc. 2(3):e00590. doi:10.1128/genomeA.00590-14

Li L, Li K, Wang Y, Chen C, Xu Y, Zhang L, Han B, Gao C, Tao F, Ma C, Xu P (2015) Metabolic engineering of *Enterobacter cloacae* for high-yield production of enantiopure (*2R,3R*)-2,3-butanediol from lignocellulose-derived sugars. Metab Eng 28:19–27. doi:10.1016/j.ymben.2014.11.010

Marquez-Villavicencio MdP, Weber B, Witherell RA, Willis DK, Charkowski AO (2011) The 3-Hydroxy-2-Butanone Pathway Is Required for Pectobacterium carotovorum Pathogenesis. PLoS ONE 6(8):e22974. doi:10.1371/journal.pone.0022974

Meldau DG, Meldau S, Hoang LH, Underberg S, Wunsche H, Baldwin IT (2013) Dimethyl disulfide produced by the naturally associated bacterium *bacillus* sp. B55 promotes *Nicotiana attenuata* growth by enhancing sulfur nutrition. Plant Cell 25:2731–2747. doi:10.1105/tpc.113.114744

Mercke P, Kappers IF, Verstappen FW, Vorst O, Dicke M, Bouwmeester HJ (2004) Combined transcript and metabolite analysis reveals genes involved in spider mite induced volatile formation in cucumber plants. Plant Physiol 135:2012–2024. doi:10.1104/pp.104.048116

Moons P, van Houdt R, Vivijs B, Michiels CW, Aertsen A (2011) Integrated regulation of acetoin fermentation by quorum sensing and pH in *Serratia plymuthica* RVH1. Appl Environ Microbiol 77:3422–3427. doi:10.1128/AEM.02763-10

Moore AJ, Moore PJ (1999) Balancing sexual selection through opposing mate choice and male competition. Proc R Soc Lond B Biol Sci 266:711–716

Moore AJ, Haynes KF, Preziosi RF, Moore PJ (2002) The evolution of interacting phenotypes: genetics and evolution of social dominance. Am Nat 160(Suppl 6):S186–S197. doi:10.1086/342899

Nielsen DR, Yoon SH, Yuan CJ, Prather KL (2010) Metabolic engineering of acetoin and meso-2,3-butanediol biosynthesis in *E. coli*. Biotechnol J 5:274–284. doi:10.1002/biot.200900279

Nout MJR, Bartelt RJ (1998) Attraction of a flying nitidulid (*Carpophilus humeralis*) to volatiles produced by yeasts grown on sweet corn and a corn-based medium. J Chem Ecol 24:1217–1239

Okubara P, Paulitz T (2005) Root defense responses to fungal pathogens: a molecular perspective. In: Lambers H, Colmer T (eds) Root physiology: from gene to function, vol 4., Plant EcophysiologySpringer, Netherlands, pp 215–226. doi:10.1007/1-4020-4099-7_11

Oliver JW, Machado IM, Yoneda H, Atsumi S (2013) Cyanobacterial conversion of carbon dioxide to 2,3-butanediol. Proc Natl Acad Sci USA 110:1249–1254. doi:10.1073/pnas.1213024110

Peralta-Yahya PP, Keasling JD (2010) Advanced biofuel production in microbes. Biotechnol J 5:147–162. doi:10.1002/biot.200900220

Rao B, Zhang LY, Sun J, Su G, Wei D, Chu J, Zhu J, Shen Y (2012) Characterization and regulation of the 2,3-butanediol pathway in *Serratia marcescens*. Appl Microbiol Biotechnol 93:2147–2159. doi:10.1007/s00253-011-3608-5

Renna MC, Najimudin N, Winik LR, Zahler SA (1993) Regulation of the *Bacillus subtilis* alsS, alsD, and alsR genes involved in post-exponential-phase production of acetoin. J Bacteriol 175:3863–3875

Robacker DC, Lauzon CR (2002) Purine metabolizing capability of *Enterobacter agglomerans* affects volatiles production and attractiveness to Mexican fruit fly. J Chem Ecol 28:1549–1563

Rochat D, Morin JP, Kakul T, Beaudoin-Ollivier L, Prior R, Renou M, Malosse I, Stathers T, Embupa S, Laup S (2002) Activity of male pheromone of *Melanesian rhinoceros* beetle *Scapanes australis*. J Chem Ecol 28:479–500

Rudrappa T, Biedrzycki ML, Kunjeti SG, Donofrio NM, Czymmek KJ, Pare PW, Bais HP (2010) The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. Commun Integr Biol 3:130–138

Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Pare PW, Kloepper JW (2003) Bacterial volatiles promote growth in *Arabidopsis*. Proc Natl Acad Sci USA 100:4927–4932. doi:10.1073/pnas.0730845100

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Pare PW (2004a) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026. doi:10.1104/pp.103.026583

Ryu CM, Murphy JF, Mysore KS, Kloepper JW (2004b) Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic virus* by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. Plant J 39:381–392. doi:10.1111/j.1365-313X.2004.02142.x

Ryu CM, Hu C-H, Locy R, Kloepper J (2005a) Study of mechanisms for plant growth promotion elicited by rhizobacteria in *Arabidopsis thaliana*. Plant Soil 268:285–292. doi:10.1007/s11104-004-0301-9

Ryu CM, Farag MA, Paré PW, Kloepper JW (2005b) Invisible signals from the underground: bacterial volatiles elicit plant growth promotion and induce systemic resistance. Plant Pathol J 21:7–12

Ryu CM, Choi HK, Lee CH, Murphy JF, Lee JK, Kloepper JW (2013) Modulation of quorum sensing in acylhomoserine lactone-producing or -degrading tobacco plants leads to alteration of induced systemic resistance elicited by the rhizobacterium *Serratia marcescens* 90-166. Plant Pathol J 29:182–192. doi:10.5423/PPJ.SI.11.2012.0173.R2

Song GC, Ryu CM (2013) Two Volatile organic compounds trigger plant self-defense against a bacterial pathogen and a sucking insect in cucumber under open field conditions. Int J Mol Sci 14:9803–9819. doi:10.3390/ijms14059803

Ton J, D'Alessandro M, Jourdie V, Jakab G, Karlen D, Held M, Mauch-Mani B, Turlings TC (2007) Priming by airborne signals boosts direct and indirect resistance in maize. Plant J 49:16–26. doi:10.1111/j.1365-313X.2006.02935.x

Ui S, Okajima Y, Mimura A, Kanai H, Kudo T (1997) Molecular generation of an *Escherichia coli* strain producing only the meso-isomer of 2,3-butanediol. J Ferment Bioeng 84:185–189. doi:10.1016/S0922-338X(97)82052-1

Velázquez-Becerra C, Macías-Rodríguez LI, López-Bucio J, Altamirano-Hernández J, Flores-Cortez I, Valencia-Cantero E (2010) A volatile organic compound analysis from *Arthrobacter agilis* identifies dimethylhexadecylamine, an amino-containing lipid modulating bacterial growth and *Medicago sativa* morphogenesis *in vitro*. Plant Soil 339:329–340. doi:10.1007/s11104-010-0583-z

Vespermann A, Kai M, Piechulla B (2007) Rhizobacterial volatiles affect the growth of fungi and *Arabidopsis thaliana*. Appl Environ Microbiol 73:5639–5641. doi:10.1128/AEM.01078-07

Weisskopf L (2013) The potential of bacterial volatiles for crop protection against phytophathogenic fungi. In: Méndez-Vilas A (ed) Microbial pathogens and strategies for combating them: science, technology and education, vol 2. Formatex Research Center, Spain, pp 1352–1363. ISBN: 978-84-942134-0-3

Wevers E, Moons P, van Houdt R, Lurquin I, Aertsen A, Michiels CW (2009) Quorum sensing and butanediol fermentation affect colonization and spoilage of carrot slices by *Serratia plymuthica*. Int J Food Microbiol 134:63–69. doi:10.1016/j.ijfoodmicro.2008.12.017

Wheatley RE (2002) The consequences of volatile organic compound mediated bacterial and fungal interactions. Antonie Van Leeuwenhoek 81:357–364

Xiao Z, Xu P (2007) Acetoin metabolism in bacteria. Crit Rev Microbiol 33:127–140. doi:10.1080/10408410701364604

Xu Y, Chang P, Liu D, Narasimhan ML, Raghothama KG, Hasegawa PM, Bressan RA (1994) Plant defense genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell 6:1077–1085. doi:10.1105/tpc.6.8.1077

Xu Y, Chu H, Gao C, Tao F, Zhou Z, Li K, Li L, Ma C, Xu P (2014) Systematic metabolic engineering of *Escherichia coli* for high-yield production of fuel bio-chemical 2,3-butanediol. Metab Eng 23:22–33. doi:10.1016/j.ymben.2014.02.004

Yang T, Rao Z, Zhang X, Xu M, Xu Z, Yang S-T (2013) Improved production of 2,3-butanediol in *Bacillus amyloliquefaciens* by over-expression of glyceraldehyde-3-phosphate dehydrogenase and 2,3-butanediol dehydrogenase. PLoS ONE 8:e76149. doi:10.1371/journal.pone.0076149

Yu Q, Tang C, Kuo J (2000) A critical review on methods to measure apoplastic pH in plants. Plant Soil 219:29–40

Zeng AP, Sabra W (2011) Microbial production of diols as platform chemicals: recent progresses. Curr Opin Biotechnol 22:749–757. doi:10.1016/j.copbio.2011.05.005

Zhang X et al (2013) Mutation breeding of acetoin high producing *Bacillus subtilis* blocked in 2,3-butanediol dehydrogenase. World J Microbiol Biotechnol 29:1783–1789. doi:10.1007/s11274-013-1339-8

Zou C, Li Z, Yu D (2010) *Bacillus megaterium* strain XTBG34 promotes plant growth by producing 2-pentylfuran. J Microbiol 48:460–466. doi:10.1007/s12275-010-0068-z