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Research Letter

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Analysis of a new cluster of genes involved in the synthesis of the unique volatile organic compound sodorifen of *Serratia plymuthica* 4Rx13

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One sentence summary: A new cluster of four genes is assigned to the biosynthesis of the volatile sodorifen of *Serratia plymuthica*.

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ABSTRACT

The rhizobacterium *Serratia plymuthica* 4Rx13 emits the novel and unique volatile sodorifen ($C_{16}H_{26}$), which has a polymethylated bicyclic structure. Transcriptome analysis revealed that gene *SOD_c20750* (annotated as terpene cyclase) is involved in the biosynthesis of sodorifen. Here we show that this gene is located in a small cluster of four genes (*SOD_c20750* – *SOD_c20780*), and the analysis of the knockout mutants demonstrated that *SOD_c20760* (annotated as methyltransferase) and *SOD_c20780* (annotated as isopentenyl pyrophosphate (IPP) isomerase) are needed for the biosynthesis of sodorifen, while a sodorifen-negative phenotype was not achieved with the *SOD_c20770* (annotated as deoxy-xylulose-5-phosphate (DOXP) synthase) mutant. Altogether, the function of this new gene cluster was assigned to the biosynthesis of this structurally unusual volatile compound sodorifen.

Keywords: *Serratia plymuthica*; sodorifen; volatile organic compound (VOC); bacterial terpene cyclase; bacterial methyltransferase; bacterial DOXP synthase

INTRODUCTION

Bacteria are a rich source of natural compounds, which often function as antibiotics. For a long time, volatile natural compounds have been neglected, but in the past decade, it was shown that a wealth of volatile compounds is emitted by bacteria (Effmert *et al.* 2012). Such volatile compounds are characterized by their molecular weights of less than 300 Da, high vapor pressures, low boiling points and low polarities. Due to these features, these air-borne signals exhibit the potential to act as infochemicals in inter- and intraspecific communication in different habitats even over long distances, although often their biological and ecological functions remain elusive (Wenke, Kai and Piechulla 2010; Effmert *et al.* 2012; Wenke *et al.* 2012).

The volatile organic compounds (VOCs) released by bacteria can be part of a very complex mixture, e.g. *Serratia plymuthica* 4Rx13 emits up to 100 compounds (Kai *et al.* 2010), or simple VOC profiles. Many emitted compounds are well known and are for example registered in the mVOC database (Lemfack *et al.* 2014), but novel compounds with unknown structures are also frequently found. The VOC profile of *S. plymuthica* 4Rx13 exhibited a dominant compound with a unique structure ($C_{16}H_{26}$) previously elucidated by us (Von Reuss *et al.* 2010). This compound was named sodorifen and was only emitted by a few *S. plymuthica* isolates (Weise *et al.* 2014). It has an extraordinary structure composed of a 5- and 6-ring, where every carbon atom of the skeleton is substituted with either a methyl or a methylene group. Due to this unusual structure, a prediction of its biosynthesis

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and chemical classification remained elusive for a long time. Recently, we successfully isolated a candidate gene (*SOD_c20750*), which was unequivocally shown by knockout mutagenesis and plasmid-assisted complementation to be involved in the biosynthesis of sodorifen (Domik *et al.* 2016). This gene was annotated as a terpene synthase, suggesting that sodorifen is a terpene. Although this achievement was a major step forward in elucidating the biosynthesis, it was obvious that the complicated structure of sodorifen cannot be synthesized by just one enzyme. Therefore, we used *SOD_c20750* as an entry to search for additional genes involved in the biosynthesis of sodorifen. The results of this strategy are presented in this paper.

MATERIALS AND METHODS

Bacterial strains, media and growth conditions

Isolation and growth of *Serratia plymuthica* 4Rx13 and *S. plymuthica* AS9 were described in Domik *et al.* (2016).

Volatile collection and analysis

Bacteria were cultivated in VOC-collection systems (Kai *et al.* 2010) to trap volatile compounds of the headspace of the bacterial culture. The VOCs were eluted in intervals of 24 h and analyzed with the Shimadzu GC/MS-QP500 (Kyoto, Japan) as described in Domik *et al.* (2016). To determine the structure of the volatile compounds, the detected mass spectra were compared with data of the NIST107 library.

Polymerase chain reaction, mutagenesis and plasmid-assisted complementation of mutants

A complete list of primers can be found in Table S1, Supporting Information. Primers were designed according to the gene properties (temperature, GC content and secondary structures) and were usually 21 nucleotides long, with the exception of the oligonucleotides for mutagenesis, which were 72 nucleotides long. The amplification of DNA fragments by polymerase chain reaction (PCR) was described in Domik *et al.* (2016). Mutagenesis was performed with the 'Quick and Easy Gene Deletion Kit' by Red/ET Recombination (Gene Bridges, Heidelberg, Germany), by introducing an antibiotic cassette via homologous recombination. The ca. 1600-bp DNA construct was obtained by high-fidelity PCR, eluted from the gel (NucleoSpin Gel and PCR Clean-up; Macherey-Nagel, Düren, Germany), and then integrated into the target gene to cause its dysfunction. The helper plasmid pRed/ET was required to integrate the functional cassette by homologous recombination. The plasmid DNA was introduced through electroporation into competent cells of *S. plymuthica* 4Rx13 (Untergasser 2008).

The inactivated gene was restored through the introduction of a plasmid containing the wild-type gene (amplified by PCR), the 500-bp upstream region of the gene *SOD_c20780* (in frame) and restriction sites of EcoRI and BamHI, altogether cloned into the vector pJET (CloneJET PCR Cloning Kit; Thermo Fisher Scientific, Waltham, USA). The modified pJET plasmid was introduced into chemically competent cells of *Escherichia coli* XL-1 Blue by electroporation (pulse control 200 Ω, capacity 25 μF and voltage of 2500 V). Immediately after the pulse cells were incubated in pre-warmed medium (0.5% yeast extract, 2% trypton, 10 mM NaCl, 2.5 mM KCl + 1 M $MgCl_2$, 1 M $MgSO_4$ and 1 M glucose; Carl Roth GmbH, Karlsruhe, Germany) at 37°C for 4 h (170 rpm), plated onto solid medium supplemented with kanamycin (50 μg ml^{-1}, Roth, GmbH, Karlsruhe, Germany) and the VOC profile was monitored.

Transcriptome analysis

The transcriptome sequencing approach of the sodorifen producer *S. plymuthica* 4Rx13 and the non-producer *S. plymuthica* AS9 was described in Domik *et al.* (2016). Differentially expressed genes were analyzed via the software Transcriptome Viewer (TraV) (Dietrich, Wiegand and Liesegang 2014). Transcriptional activity was represented as nucleotide activities per kilobase of exon model per million mapped reads (NPKM).

RESULTS

Analysis of the genes of the sodorifen cluster

The inactivation of the gene *SOD_c20750* revealed a sodorifen-negative phenotype; thus, this was the first gene found to be involved in the sodorifen biosynthesis (Domik *et al.* 2016). To locate additional biosynthetic genes, the position in the genome was studied in detail using the software antiSMASH (Medema *et al.* 2011). Subsequently, a small cluster of four genes (*SOD_c20750*, *SOD_c20760*, *SOD_c20770* and *SOD_c20780*) was identified (Fig. 1). The cluster is embedded upstream by the gene *SOD_c20740*, which was annotated as a regulator for the araBAD and araFGH operon, and other genes involved in the transport and catabolism of L-arabinose. The downstream gene *SOD_c20790* was annotated as a putative oxidoreductase. Both genes, *SOD_c20740* and *SOD_c20790*, are orientated in opposite direction to the cluster genes.

The expression levels of all four genes of the sodorifen cluster from the transcriptome analysis (Domik *et al.* 2016) were significantly above the expression levels of housekeeping genes and were higher in the sodorifen producer *Serratia plymuthica* 4Rx13 than in the non-producer *S. plymuthica* AS9 (Fig. 2). Furthermore, the transcriptome analysis suggested that all four genes with individual lengths of I: 540 bp, II: 1839 bp, III: 978 bp and IV: 1155 bp, are co-transcribed resulting in an expected transcript with a total length of 4.5 kb, which was verified by RT-PCR (Fig. S1, Supporting Information). This result favored the hypothesis that the three additional genes of this cluster also may be involved in sodorifen biosynthesis.

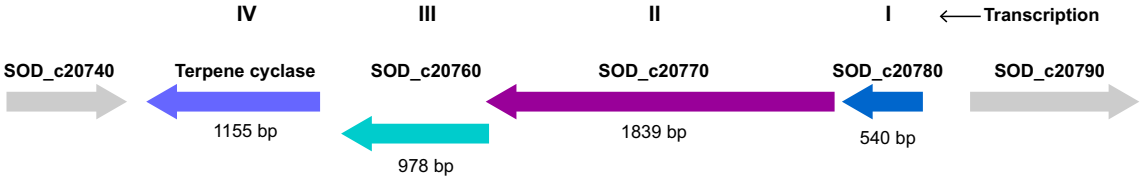

Figure 1. Localization of the *SOD_c20750* (terpene cyclase) within the genome of *S. plymuthica* 4Rx13. The terpene cyclase forms a cluster together with three additional genes, which are positioned in antisense direction within the genome of *S. plymuthica*. Lengths of genes are indicated in bp. The cluster is bordered by genes, which are in sense orientation.

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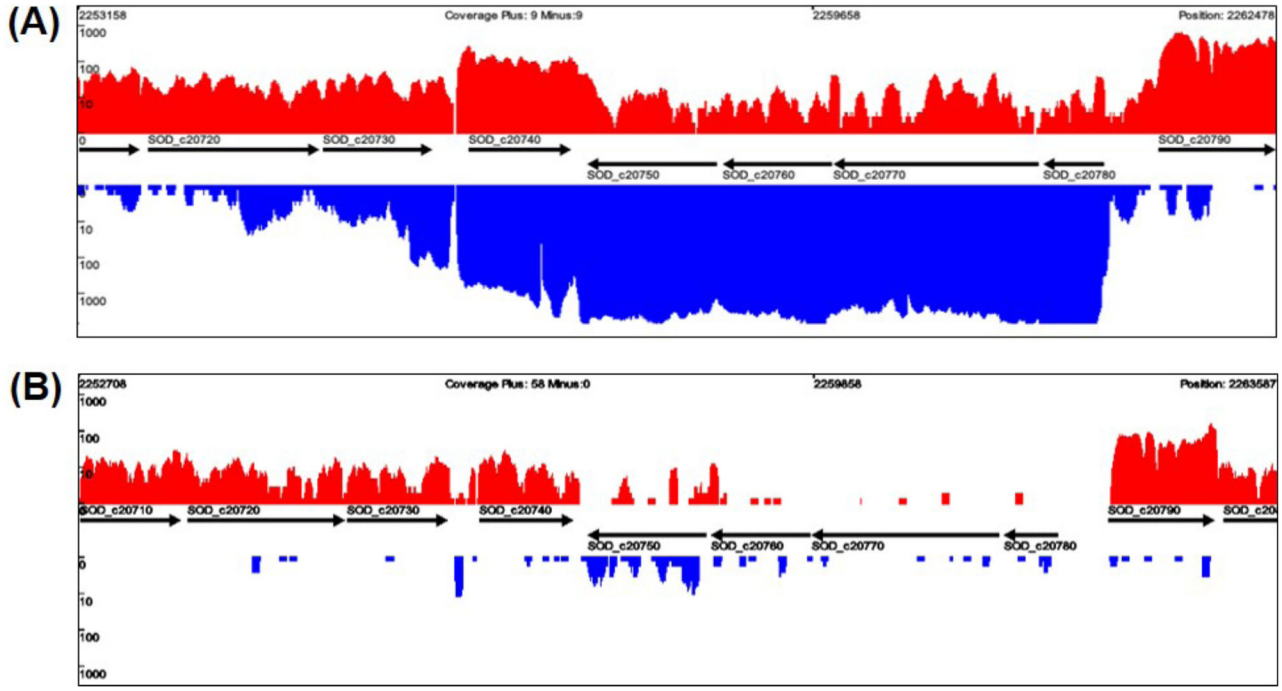

Figure 2. Visualization of the expression of the genes of the sodorifen cluster of *S. plymuthica* 4Rx13. RNA was extracted from 48-h cultures. The transcriptional activity of the genes of the sodorifen cluster (*SOD_c20750–SOD_c20780*) on the negative strand is shown in blue, while the transcriptional activity on the positive strand is shown in red. Each black arrow indicates an open reading frame. This graph was obtained using the software TraV (Dietrich, Wiegand and Liesegang 2014). The transcriptional activity was measured in NPKM and the plotted data are presented in a logarithmic scale. **(A)** *Serratia plymuthica* 4Rx13 and **(B)** *S. plymuthica* AS9.

Knockout mutagenesis of the sodorifen cluster genes

The genes *SOD_c20760*, *SOD_c20770* and *SOD_c20780* of the cluster were inactivated and VOC profiles of resulting mutants were analyzed. The knockout mutants of *SOD_c20760* and *SOD_c20780* did not emit sodorifen, while the mutant of *SOD_c20770* still emitted sodorifen (Fig. 3). As expected, the inactivation of the adjacent gene *SOD_c20790* also did not alter the sodorifen phenotype (Table S2, Supporting Information). For each mutant it was ensured that the resistance cassette was stable and correctly integrated into the target gene (Figs S2 and S3, Supporting Information). Furthermore, the involvement of the genes *SOD_c20760* and *SOD_c20780* in sodorifen biosynthesis was verified by plasmid-assisted complementation. The wild-type phenotypes of these mutants were successfully restored by introducing a recombinant plasmid into each mutant (Fig. S4, Supporting Information). The emission of sodorifen (#2) was detectable but significantly lower compared to the wild type. The sodorifen-negative phenotype of the mutants with the inactivated genes *SOD_c20760* (annotated as a methyltransferase), *SOD_c20780* (annotated as an isopentenyl pyrophosphate (IPP) isomerase) and *SOD_c20750* (terpene cyclase, Domik *et al.* 2016) supported the hypothesis that the genes of this cluster are indispensable for the sodorifen biosynthesis.

The sodorifen emission phenotype of the knockout mutant *SOD_c20770*, which most likely encodes a 1-deoxy-D-xylulose 5-phosphate (DOXP) synthase (*dxs*), was a surprising result. It was hypothesized that functionally homologous genes in the *S. plymuthica* 4Rx13 genome compensated the inactivated gene. One possible candidate for a homologous gene is *SOD_c09180*. It had 52.78% identity at the amino acid level with the *SOD_c20770* gene, and high accordance was found within the protein domains (Fig. S5, Supporting Information). To investigate the role of the *SOD_c09180* gene, a knockout mutant was generated. This mutant was still able to produce sodorifen, but the amount was significantly reduced to 29% compared to the wild-type level (Fig. 4A). Also the double knockout mutant (Δ*SOD_c20770* and Δ*SOD_c09180*) was synthesizing sodorifen, however the level was reduced to 12.5% compared to the wild type (Fig. 4B). There are two possible explanations for these results; either the *dxs* gene is not needed for the biosynthesis of sodorifen, or additional gene(s) substitute(s) the function of both genes. Subsequently, an annotated farnesyl diphosphate synthase (*SOD_c09190*) and a putative oxidoreductase YajO (*SOD_c09170*) were investigated. The generation of a mutant of the gene *SOD_c09190* led to a lethal phenotype, while Δ*SOD_c09170* emitted sodorifen like the wild type (Fig. S6, Supporting Information; Table S2, Supporting Information).

Thus, it is concluded that the enzymes encoded by the three genes of this sodorifen cluster have a function in the terpenoid metabolism and strongly support that sodorifen is a terpene, which most likely is methylated by the methyltransferase of the sodorifen cluster.

Presence of the sodorifen cluster in other bacterial species

A search for the presence of this sodorifen cluster was performed by using the online software antiSMASH (Medema *et al.* 2011). The sodorifen cluster of *S. plymuthica* 4Rx13 is present in closely related isolates belonging to the same species, including the sodorifen-producer isolate V4 and the non-producer isolate AS9 (Fig. 5). The different *S. plymuthica* isolates shared very high sequence identities at the amino acid level of all genes of the sodorifen cluster (>95%) and a maximum of four amino acid differences was noticeable between the sodorifen-producer 4Rx13, V4 and HRO-C48 isolates and the non-producer isolate AS9

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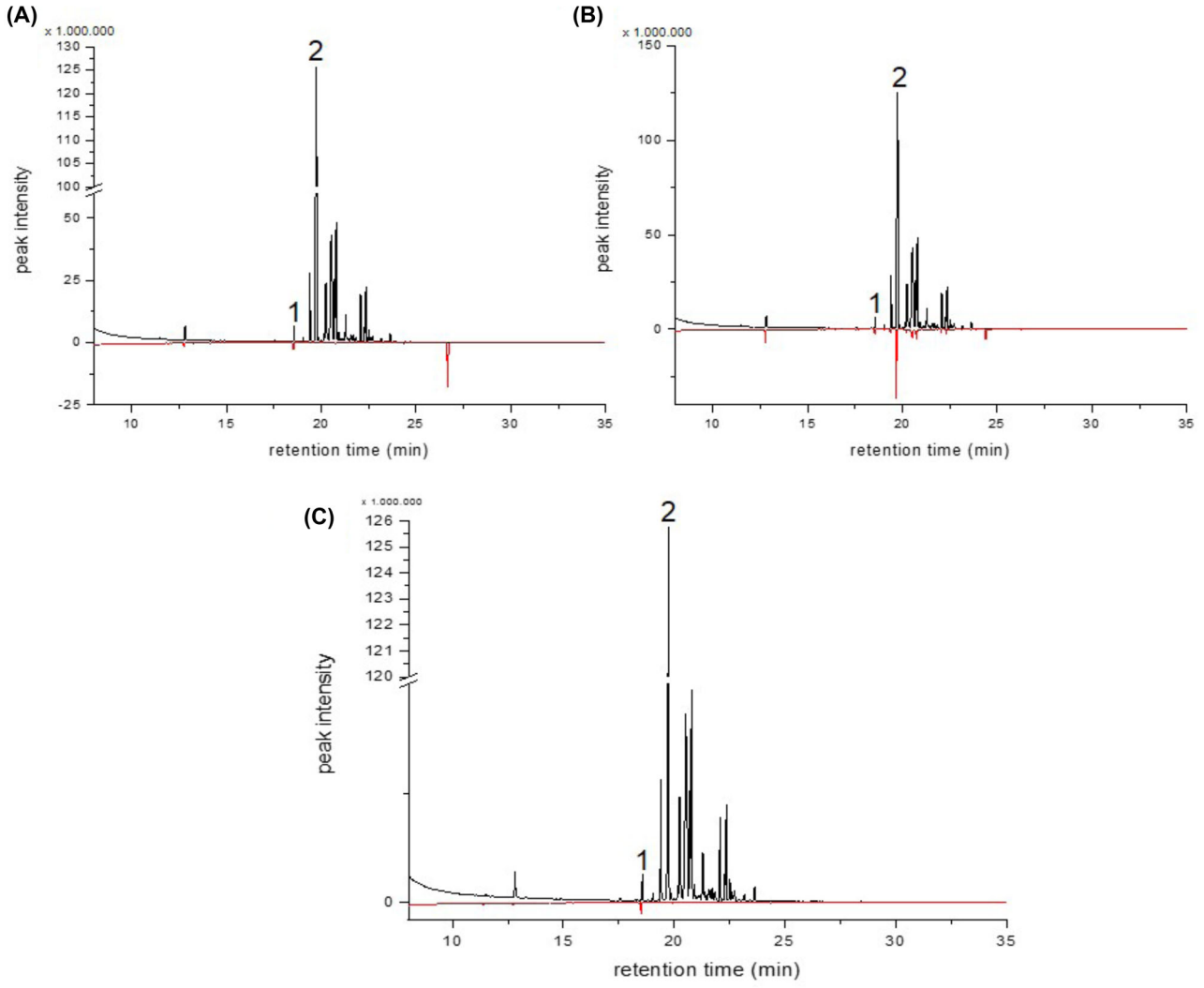

Figure 3. Analysis of the knockout mutants of the sodorifen cluster of *S. plymuthica* 4Rx13. (**A**) Mutant of *SOD_c20760*; (**B**) mutant of *SOD_c20770*; (**C**) mutant of *SOD_c20780*. Typical VOC profiles of the mutants (red lines) and the wild type (black lines) are shown when grown in complex medium (48 h) ($n = 2$). Compounds were identified by comparison of the mass spectra with those in the Nist107 library. Peak #1: internal standard nonyl actetate (peak corresponds to 5 ng), peak #2: sodorifen.

(Table 1). Comparison of the cluster structure also revealed that three out of the four genes are present in *Streptomyces tsukubaensis* (Fig. 5). Apparently, the IPP isomerase is missing, while the methyltransferase is 60 bp shorter and the *dxs* gene is 582 bp shorter than in *S. plymuthica* 4Rx13. In *Pseudomonas chlororaphis*, all four genes of the cluster are present, but they differ in length compared to the 4Rx13 isolate, the methyltransferase is 12 bp shorter, the *dxs* gene is 6 bp and the IPP isomerase is 290 bp longer. *Burkholderia pyrrocinia* contained a hypothetical protein with only 41% identity to the terpene cyclase of *S. plymuthica* 4Rx13, while the methyltransferase (64%) and DOXP synthase (62%) shared quite substantial identities, although these genes are not organized in a cluster (Table 1). A gene with homology to the putative IPP isomerase of *S. plymuthica* 4Rx13 was not found in *B. pyrrocinia*.

DISCUSSION

Analysis of the sodorifen cluster genes

The structure of the volatile natural compound sodorifen is very unusual which made the prediction of its biosynthetic origin very difficult. The identification and isolation of a gene encoding a terpene cyclase (*SOD_c20750*) (Domik *et al.* 2016) provided a handle for directed search for additional genes involved in the biosynthesis of sodorifen. Here we present that this gene is embedded in a cluster of four genes. Inactivation of the cluster genes by knockout mutagenesis provided evidence that they are essential for the sodorifen biosynthesis and clearly indicate that sodorifen derives from the terpenoid metabolism, due to its molecular formula $C_{16}H_{26}$ it is expected to be a sesquiterpene. The methyltransferase of the cluster is most likely involved in synthesizing the polymethylated sodorifen.

The discovery of two distantly related *dxs* isogenes demonstrated a new complexity in the genome of *Serratia plymuthica* 4Rx13. *Dxs* isogenes have previously been described in roots colonized by arbuscular mycorrhizal fungi, which have distinct functions and expression levels (Walter *et al.* 2013). The DOXP synthase 1 is responsible for housekeeping functions of the primary metabolism, while DOXP synthase 2 is associated with the synthesis of special isoprenoids of the secondary metabolism, although each gene can (partially) complement the function of the other. Based on the specific function of the two DOXP

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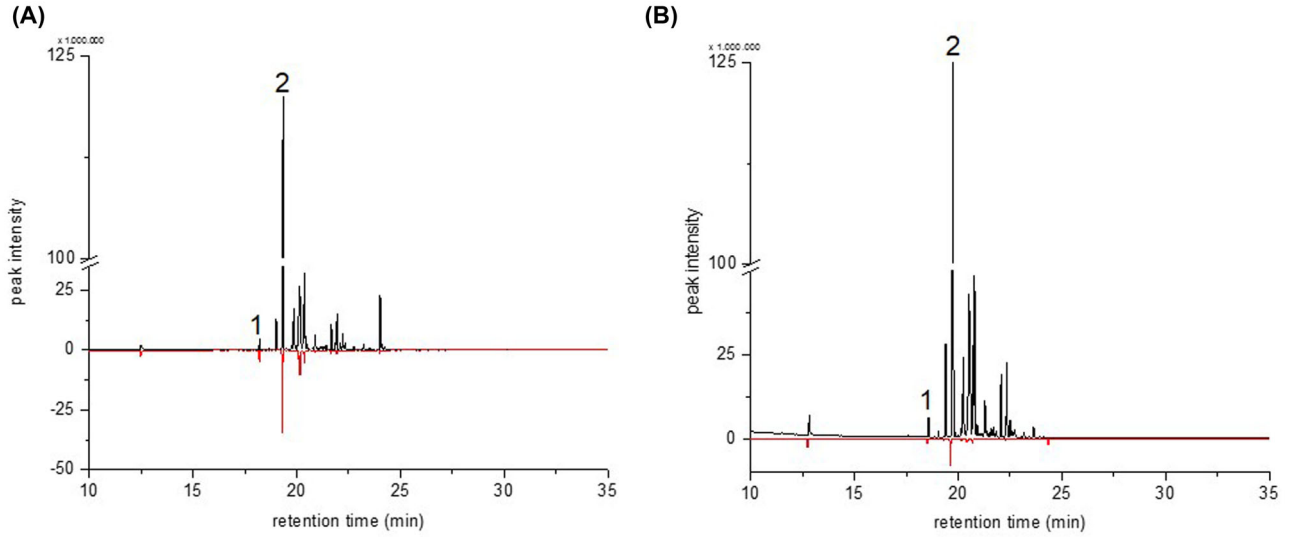

Figure 4. Volatile spectra of *dxs* gene mutants in comparison to the wild type for *S. plymuthica* 4Rx13. (**A**) Mutant of SOD_c09180, (**B**) double mutant *SOD_c20770*::FRT-neo-cassette, Δ*SOD_c09180*. Typical VOC profiles of the knockout mutants of homologous *dxs* genes (red) and wild type (black) are shown when grown in complex medium (48 h) ($n = 2$). Compounds were identified by comparison of the mass spectra with those in the Nist107 library. Peak #1: internal standard nonyl actetate (peak corresponds to 5 ng), peak #2: sodorifen.

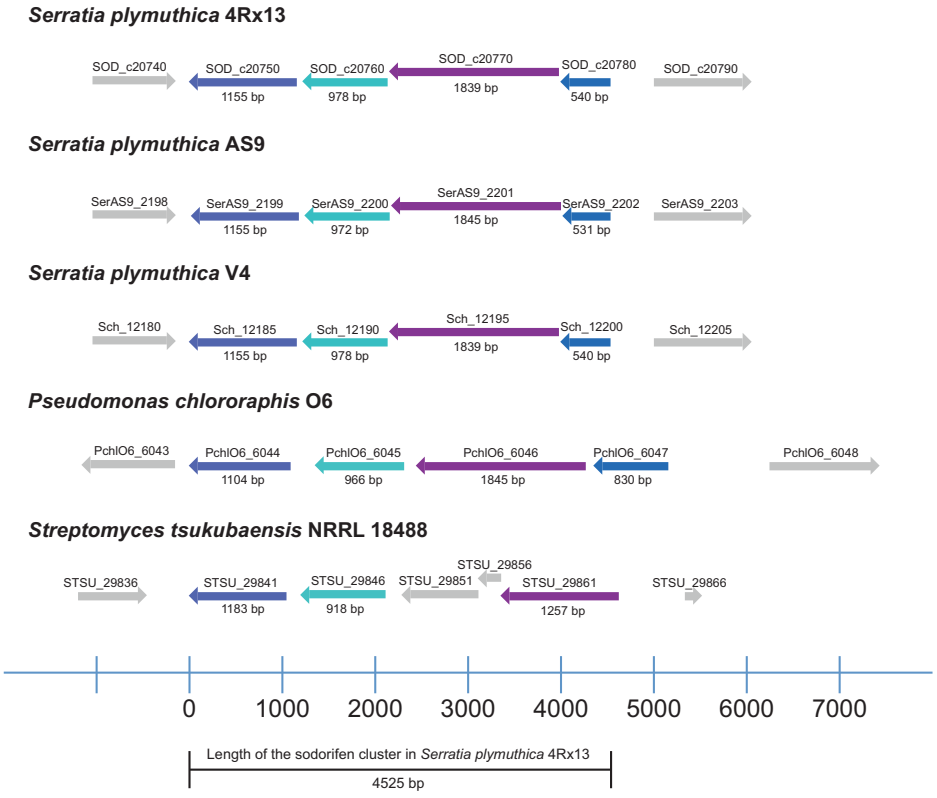

Figure 5. The presence of the sodorifen cluster genes in other bacteria. The search for the sodorifen cluster was performed using the online tool antiSMASH. Homologous genes are presented in the same color. Lengths of genes are indicated in base pairs. The cluster is bordered by genes, which are in opposite orientation (grey).

synthases, a difference in transcript levels was expected. In *S. plymuthica* 4Rx13 it was found that the expression of the *dxs* gene (*SOD_c09190*) outside the cluster matched the expression profile of a housekeeping gene (NPKM values below 100), while the *dxs* gene (*SOD_c20770*) within the sodorifen cluster reached 10-fold higher expression levels, suggesting that this gene is probably involved in the production of the secondary metabolite sodorifen.

Inactivation of the DOXP synthase (*SOD_c20770*) as well as the complementing gene *SOD_c09180* led to a significant reduction of sodorifen emission, but not to a non-producing phenotype. Also, the double knockout mutant produced sodorifen

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Table 1. Amino acid identities of the sodorifen cluster genes in *S. plymuthica* and other species.

Gene/ organism	Terpene cyclase	Methyl transferase	DOXP	Isopentenyl diphosphate (IPP)-isomerase	Amino acid alterations compared to the terpene cyclase of *S. plymuthica* 4Rx13
S. plymuthica 4Rx13	100%	100%	100%	100%	–
S. plymuthica AS9	99%	97%	95%	96%	M14T; E328Q; N357S; Y381H
S. plymuthica V4	99%	98%	98%	99%	A275T; N375S; Y381H; G382S
S. plymuthica HRO-C48	99%	98%	98%	99%	A275T; N375S; Y381H; G382S
P. chlororaphis O6	43%	59%	65%	50%	<40
St. tsukubaensis NRRL18488	41%	52%	50%	–	<40
B. pyrrocinia	41%	64%	62	–	<40

suggesting that a third (or more) gene(s) replaces their functions, given that *SOD_c20770* is involved in the biosynthesis. Further analysis of the genome location around *SOD_c09180* revealed a small cluster of three genes, all of which have the same transcription direction and appeared to belong to the DOXP pathway. This cluster encoded a gene for a farnesyl diphosphate synthase (*ispA* gene, *SOD_c09190*) and a putative oxidoreductase (*YajO*, *SOD_c09170*). These genes had the same structure as homologous genes in *Escherichia coli*, and Sponsel (2001) speculated that the proximity of *dxs* to *ispA* is due to their functional relationship and involvement in the same pathway. It was not possible to generate a mutant lacking the farnesyl diphosphate synthase (*ispA*) (Table S2, Supporting Information) indicating its unique and essential requirement. The knockout of the oxidoreductase (*YajO*) had no effect on sodorifen emission (Fig. S6B, Supporting Information), most likely due to the presence of multiple putative oxidoreductases within the genome of *S. plymuthica* 4Rx13.

Since homologous genes of the *dxs* were found in *S. plymuthica* 4Rx13, the genome was also rescreened for homologous genes of the other sodorifen cluster genes. The terpene cyclase is unique in *S. plymuthica* 4Rx13 (Domik *et al.* 2016). Another annotated methyltransferase (*SOD_c00380*) was detected but had no significant similarity or domain correlations to the enzyme in the sodorifen cluster. Therefore, it is most likely that the putative methyltransferase of the cluster is specifically involved in methylation reactions to reveal the polymethylated sodorifen molecule. A homologous gene of the IPP isomerase (*SOD c20780*) was also found. This putative orthologous *SOD_c20170* appeared to have a similar length and similar protein domains resulting in an overall identity of 37.78% at the amino acid level, however the knockout mutant of the latter produced sodorifen, which suggested that *SOD_c20170* does not functionally complement *SOD_c20780* (Fig. S6, Supporting Information).

Organization of the sodorifen cluster

Sodorifen production is—at least partially—realized by a cluster of four genes in *S. plymuthica* 4Rx13. A cluster with an analogous arrangement of genes was found in *Streptomyces tsukubaensis* NRRL 18488 and *Pseudomonas chlororaphis* O6. Despite the presence of these similar gene arrangements, it is presently unknown whether both species are sodorifen emitters, because the gene regulation could be remarkably different. This was found to be relevant for *St. griseus,* which harbors the complete set of genes for geosmin biosynthesis, but almost no detectable production was observed (Yamada *et al.* 2015). Another example is provided by *St. avermitilis,* which contained a biosynthesis cluster for the production of pentalenolactone; however, when this cluster was integrated into the non-producer *St. lividans*, it did not produce pentalenolactone but pentalenic acid (Tetzlaff *et al.* 2006).

The sodorifen cluster has some similarity to the terpene synthase cluster of *St. clavuligerus*, which also consisted of a terpene cyclase and a methyltransferase, flanked by an oxidoreductase in the opposite orientation (Hu *et al.* 2011). Another two-gene operon was found in *St. coelicolor*, which is responsible for the biosynthesis of the volatile terpenoid compound methylisoborneol (Wang and Cane 2008). Finding the cluster in *S. plymuthica* 4Rx13 was an important step forward in elucidating the biosynthetic pathway of the novel compound sodorifen, but these four genes are certainly not sufficient to synthesize such a complicated structure as sodorifen. Thus, further investigations are essential to locate additional genes that are involved in sodorifen biosynthesis. This is not a trivial task given our limited knowledge about the general organization of the terpenoid biosynthetic pathways in bacteria (Tetzlaff *et al.* 2006). It is possible that the remaining genes will be scattered within the genome (DeJong *et al.* 2006). The putative oxidoreductase, putative serine protease and an adenosine deaminase, which are located in direct proximity upstream of the sodorifen cluster as well as the genes downstream of the cluster which belong to the arabinose biosynthesis pathway are excluded from being involved in the biosynthesis of sodorifen.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSLE online.

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Conflict of interest. None declared.

REFERENCES

DeJong JM, Liu Y, Bollon AP *et al.* Genetic engineering of taxol biosynthetic genes in *Saccharomyces cerevisiae*. *Biotechnol Bioeng* 2006;**93**:212–24.

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Dietrich S, Wiegand S, Liesegang H. TraV: a genome context sensitive transcriptome browser. *PloS One* 2014;**9**:e93677.

Domik D, Thürmer A, Daniel R *et al.* A terpene synthase is involved in the synthesis of the unique volatile organic compound sodorifen of *Serratia plymuthica* 4Rx13. *Front Microbiol* 2016;**7**:737.

Effmert U, Kalderás J, Warnke R *et al.* Volatile mediated interactions between bacteria and fungi in the soil. *J Chem Ecol* 2012;**38**:665–703.

Hu Y, Chou WK, Hopson R *et al.* Genome mining in *Streptomyces clavuligerus*: expression and biochemical characterization of two new cryptic sesquiterpene synthases. *Chem Biol* 2011;**18**:32–7.

Kai M, Crespo E, Cristescu SM *et al.* *Serratia odorifera*: analysis of volatile emission and biological impact of volatile compounds on *Arabidopsis thaliana*. *Appl Microbiol Biot* 2010;**88**:965–76.

Lemfack MC, Nickel J, Dunkel M *et al.* mVOC: a database of microbial volatiles. *Nucleic Acids Res* 2014;**42**:D744–8.

Medema MH, Blin K, Cimermancic P *et al.* AntiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res* 2011;**39**:339–46.

Sponsel VM. The deoxyxylulose phosphate pathway for the biosynthesis of plastidic isoprenoids: early days in our understanding of the early stages of gibberellin biosynthesis. *J Plant Growth Regul* 2001;**20**:332–45.

Tetzlaff CN, You Z, Cane DE *et al.* A gene cluster for biosynthesis of the sesquiterpenoid antibiotic pentalenolactone in *Streptomyces avermitilis*. *Biochemistry* 2006;**45**:6179–86.

Untergasser A. *Preparation of Electro-Competent Cells*. Untergasser's Lab, 2008, http://www.untergasser.de/lab/protocols/competent_cells_electro_v1_0.htm (June 2016).

Von Reuss SH, Kai M, Piechulla B *et al.* Octamethylbicyclo [3.2. 1] octadienes from the rhizobacterium *Serratia odorifera*. *Angew Chem Int Edit* 2010;**49**:2009–10.

Walter MH, Floss DS, Paetzold H *et al.* Control of plastidial isoprenoid precursor supply: divergent 1-deoxy-d-xylulose 5-phosphate synthase (dxs) isogenes regulate the allocation to primary or secondary metabolism. In: Bach TJ, Rohmer M (eds). *Isoprenoid Synthesis in Plants and Microorganisms: New Concepts and Experimental Approaches*. New York: Springer, 2013, 251–70.

Wang CM, Cane DE. Biochemistry and molecular genetics of the biosynthesis of the earthy odorant methylisoborneol in *Streptomyces coelicolor*. *J Am Chem Soc* 2008;**130**:8908–9.

Weise T, Thürmer A, Brady S *et al.* VOC emission of various Serratia species and isolates and genome analysis of *Serratia plymuthica* 4Rx13. *FEMS Microbiol Lett* 2014;**352**:45–53.

Wenke K, Kai M, Piechulla B. Belowground volatiles facilitate interactions between plant roots and soil organisms. *Planta* 2010;**231**:499–506.

Wenke K, Weise T, Warnke R *et al.* Bacterial volatiles mediating information between bacteria and plants. In: Witzany G, Baluška F (eds). *Biocommunication of Plants*. Berlin, Heidelberg: Springer, 2012, 327–47.

Yamada Y, Kuzuyama T, Komatsu M *et al.* Terpene synthases are widely distributed in bacteria. *P Natl Acad Sci USA* 2015;**112**:857–62.

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