RESEARCH LETTER

VOC emission of various *Serratia* species and isolates and genome analysis of *Serratia plymuthica* 4Rx13

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Abstract

Bacteria emit a wealth of volatile organic compounds. Gas chromatography coupled to mass spectrometry analysis of five *Serratia* strains revealed ketones, dimethyl di- and trisulfide and 2-phenylethanol commonly released in this genus. The polymethylated bicyclic hydrocarbon sodorifen was uniquely released by the rhizobacterium *Serratia plymuthica* 4Rx13. Of 10 *Serratia* strains, only *S. plymuthica* isolates originating from plants grown on fields near Rostock (Germany) released this new and unusual compound. Since the biosynthetic pathway of sodorifen was unknown, the genome sequence of *S. plymuthica* 4Rx13 was determined and annotated. Genome comparison of *S. plymuthica* 4Rx13 with sodorifen non-producing *Serratia* species highlighted 246 unique candidate open reading frames.

Introduction

The genus *Serratia* is composed of facultative anaerobic, rod-shaped *Gammaproteobacteria* with peritrichous flagella (summarized in Mahlen, 2011). They appear ubiquitously in/on soil, water, plants and animals, including humans. The most commonly known human-derived species is *Serratia marcescens*. Since 1823, 15 species have been identified (Mahlen, 2011) but the taxonomy of the genus *Serratia* is still not fully clarified and many species have a long history of being renamed and redefined until they finally were accepted in the genus *Serratia* (Mahlen, 2011; Breed & Breed, 1924; Martinec & Kocur, 1961; Grimont *et al.*, 1977, 1978; Ashelford *et al.*, 2002, Manzano-Marin *et al.*, 2012).

Serratia species are characterized by their opaque appearance with either a white, pink or red color, the latter being due to the pigments prodigiosin and pyrimine. Prodigiosin (2-methyl-3-amyl-6-methoxyprodigiosene) is a non-diffusible, water-insoluble, red-colored pigment bound to the cell envelope with antibacterial, antifungal, antimalarial and antiprotozoal features (summarized in Slater *et al.*, 2003). Pyrimine (ferrorosamine A) is a water-soluble pink pigment containing iron (Fe^{2+}) (Grimont & Grimont, 2006). Beside these pigments, other secondary metabolites are produced by *Serratia* species (Fender *et al.*, 2012), e.g. carbapenem (1-carbapen-2-em-3-carboxylic acid) is a broad spectrum β-lactam antibiotic (summarized in Fineran *et al.*, 2005), the polyketide oocydin A acting against oomycetes (Matilla *et al.*, 2012) and althiomycin, which is a ribosome-inhibiting antibiotic (Gerc *et al.*, 2012). *Serratia* species also produce chitinases as well as the plant hormone indole-3-acetic acid (IAA), which influences the growth of plant pathogenic fungi (Kalbe *et al.*,

1996; Frankowski *et al.*, 2001). Due to its activities in the rhizosphere and suppression capabilities, *S. plymuthica* HRO-C48 is commercially used as a biocontrol agent (summarized in Müller *et al.*, 2008).

The potential of emitted volatile organic compounds (VOC) was recently rediscovered for *Serratia* as well as for other bacteria. Up to now, *c.* 1000 different volatiles have been shown to be released from *c.* 350 bacterial species (Effmert *et al.*, 2012; Lemfack *et al.*, 2013). *Chondromyces crocatus, Carnobacterium divergens, Streptomyces* spp. and *Serratia* spp. emit particularly complex blends. *C.* 100 compounds were found in the headspace of *S. plymuthica* 4Rx13 (previously *S. odorifera* 4Rx13) (Kai *et al.*, 2010). Its VOC profile was dominated by a major compound, which constituted *c.* 45% of the total mixture. The structure of this polymethylated volatile bicyclic hydrocarbon was recently assigned and named sodorifen (von Reuss *et al.*, 2010). Although its biological and/or ecological relevance is presently unknown, this novel and highly remarkable structure raised the question of its synthesis in other *Serratia* species. Here we present the analysis of headspace volatiles of 15 *Serratia* species/isolates originating from plants, animals, and humans from different locations. 16S rRNA gene sequences and genome comparison were used to determine the phylogenetic relationships, which were further correlated with the capability of sodorifen synthesis and the origin of the species.

Materials and methods

Bacterial strains and culture conditions

Serratia strains and isolates (listed in Supporting Information, Table S1) were grown in 6 mL liquid nutrient broth cultures (NB II; peptone from casein 3.5 g L^{-1}, peptone from meat 2.5 g L^{-1}, peptone from gelatine 2.5 g L^{-1}, yeast extract 1.5 g L^{-1}, NaCl 5 g L^{-1}) at 30 °C. Growth of the bacteria was monitored by determining living cell numbers (cfu) and optical density at 600 nm. Stocks were prepared by adding glycerol (50%) to an overnight culture and storage at −70 °C.

Analysis of volatiles produced by *Serratia* species

Collection and analysis of volatiles were performed as described by Kai *et al.* (2010). Volatiles were trapped after 24 and 48 h. Compounds were identified using commercially available authentic reference compounds (Roth, Germany) or reference compounds synthesized by Prof. Wittko Francke (University of Hamburg) and by comparison of mass spectra with the library of mass spectra of the National Institute of Standards and Technology (NIST147) and comparison of retention times and Kovats indices. Compounds emitted by the NB II medium were subtracted. Analysis of volatile emissions was performed at least twice.

Isolation of genomic DNA from *Serratia* isolates and 16S rRNA gene sequencing

Total DNA of the different *Serratia* isolates (Table S1) was extracted as recommended by the manufacturer (MasterPure™ complete DNA purification kit, Epicentre, Madison, WI). 16S rRNA genes were amplified using the forward primer 16S-08_for 5′-AGAGTTTGATCCTGGC-3′, and the reverse primer 16S-1504_rev 5′-TACCTTGTTACGACT T-3′ (Muyzer *et al.*, 1993). The PCR products were sequenced by Sanger-based technology. The quality was improved by an additional primer walking step (primer 16S-349_for 5′-TCCTACGGGAGGCAGCAGT-3′; Nadkarni *et al.*, 2002). Each PCR amplification reaction mixture (final volume 50 μL) contained 0.5 U of Bio-X-ACT™ polymerase (Bioline, Germany), 5 μL 10-fold reaction buffer (Bioline), 0.2 mM dNTPs (Roche Applied Science, Penzberg, Germany), 0.5 μM of each primer, and 50 ng of template DNA. The following thermal cycling scheme was used: initial denaturation for 30 s at 98 °C and 30 cycles of denaturation for 10 s at 98 °C, annealing for 30 s at 62 °C and extension for 45 s at 72 °C, followed by a final extension of 5 min at 72 °C.

Phylogenetic analysis (dendrogram)

The 16S rRNA gene sequences were aligned using the FastAligner utility of the ARB program (Ludwig *et al.*, 2004). The automatic alignment was verified against known secondary structures and corrected manually. The phylogenetic tree (Fig. 1) was generated using the neighbor-joining method (Saitou & Nei, 1987), incorporated in the ARB software with genetic distances computed by using the Jukes–Cantor model for multiple substitutions (Jukes & Cantor, 1969). *Escherichia coli* was used as the outgroup. The robustness of the inferred trees was evaluated by a bootstrap analysis consisting of 1000 resamplings.

Whole genome sequencing, assembly and gap closure

The isolated DNA from *S. plymuthica* 4Rx13 was used to create a 454-shotgun library following the GS rapid library protocol (Roche, Mannheim, Germany). The resulting library was sequenced using the 454 FLX pyrosequencing system (Roche 454, Branford, CT) and titanium chemistry. Two medium lanes of a titanium pico titer plate were used, resulting in 274101 shotgun reads and 93.8 Mb in total. Reads were assembled *de novo* with the 454 NEWBLER

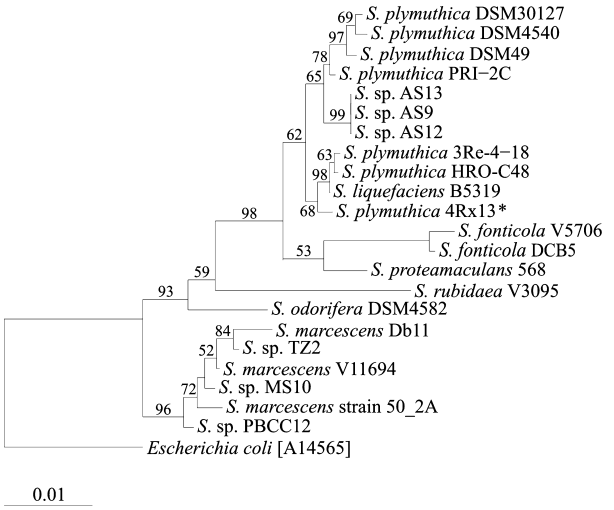

Fig. 1. 16S rRNA gene-based phylogenetic tree of *Serratia* species/isolates. Phylogenetic tree showing the relationships among *Serratia* species using the neighbor-joining method corrected by the Jukes–Cantor formula for multiple substitutions. The numbers at the nodes indicate the levels of bootstrap support based on data for 1000 replicates. *Escherichia coli* was used as outgroup. The scale bar indicates 0.01 substitution per nucleotide position. *Former *S. odorifera* 4Rx13.

assembler software v2.0 resulting in 41 contigs (> 500 bp) with an initial genome size of 5.3 Mb. In parallel, a DNA fosmid library (average insert size of 35 kb) was generated using the EpiFOS™ fosmid library production kit (Epicentre Technologies, Chicago, IL) following the instructions of the manufacturer. Fosmid inserts were end-sequenced on ABI3730xl sequencers (Applied Biosystems, Darmstadt, Germany) using the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems). The sequences of *c.* 400 recombinant fosmids were automatically processed with Pregap and aligned to the 454 contigs with GAP4 software package v4.6 (Staden *et al.*, 2000). Primer walking on fosmid inserts and PCR-based techniques were used to close remaining gaps. After sequence polishing and finishing, the coverage of the genome was *c.* 18-fold sequenced.

Genome and bidirectional BLAST analysis

Whole genome sequence alignments were done with the MUMMER software tool v3.22. The coding sequences (CDS) and open reading frames (ORFs) were predicted with the YACOP (Tech & Merkl, 2003) software tool using the ORF finders Glimmer (Delcher & Harmon, 1999), Critica (Badger & Olsen, 1999) and Z-curve (Guo & Ou, 2003). The ARTEMIS software tool was used for the manual correction of all CDS (Rutherford *et al.*, 2000). The protein sequences encoded by *S. plymuthica* 4Rx13 were used for a bidirectional BLAST comparison with whole genome proteins of *S. plymuthica* AS9, *S. plymuthica* PRI-2C, *S. odorifera* DSM4582, *S. marcescens* Db11, and *S. proteamaculans* 568. One phage-region (genomic coordinates 4060600–4104861), genomic islands (Table S2) were identified with PHAST (http://phast.wishartlab.com/index.html) and ISLANDVIEWER (http://www.pathogenomics.sfu.ca/islandviewer/genome_submit.php), and potential secondary metabolite clusters were analyzed with ANTISMASH (http://antismash.secondarymetabolites.org/) (Medema *et al.*, 2011; Table S5).

Results and discussion

Taxonomic analysis of the genus *Serratia*

Various *Serratia* species originating from plants, animals and humans from different locations (Table S1) were phylogenetically analyzed. The results of 16S rRNA gene sequences and those sequences of *Serratia* species available in the NCBI database were aligned and a phylogenetic tree was constructed using the neighbor-joining method with Jukes–Cantor correction (Fig. 1). The dendrogram separated the *S. marcescens* clade from the *S. odorifera* and *S. rubidaea* clades. *Serratia fonticola* and *S. proteamaculans* clustered together and were distinct from the *S. plymuthica* clade. These results were congruent with the phylogenetic tree published by Dauga *et al.* (1990) and Mahlen (2011). Within the *S. plymuthica* clade we found two main branches; branch I comprised the isolates DSM30127, DSM4540, DSM49, PRI-2C, AS9, AS12 and AS13, and branch II included the isolates 3Re4-18, HRO-C48, 4Rx13 and *S. liquefaciens* B5319. The isolate 4Rx13 was previously identified as *S. odorifera* (Berg *et al.*, 2002). However, based on our 16S rRNA gene sequencing and whole genome bidirectional BLAST analysis (Fig. 1, Table S3) this isolate belongs to the *S. plymuthica* clade. Therefore, this isolate will be renamed *S. plymuthica* 4Rx13. The 16S rRNA gene analysis also placed the isolate *S. liquefaciens* B5319 into the *S. plymuthica* clade; however, in this case we suggest no renaming because whole genome comparison or other markers supporting the new categorization are not available at present.

General genome features

To obtain further insights, the genome of *S. plymuthica* 4Rx13 was completely sequenced by a combination of 454- and Sanger-sequencing (CP006250, CP006251; Fig. 2). The chromosome of *S. plymuthica* 4Rx13 is 5.33 Mb and the plasmid has a size of 75.72 kb. The chromosome and the plasmid harbored 4742 and 58 putative ORFs, respectively. The G/C content of 56.2% of

S. plymuthica 4Rx13 and the genome size are very similar to other *Serratia* species (Table S3). Whole genome sequence alignment revealed 94–96% sequence identity with other *S. plymuthica* isolates present in the NCBI database (CP002773, AJTB01000001-AJTB01000104). Lower identities (85–89%) were observed to other species of the genus, *S. odorifera* DSM4582 (ADBY01000000-ADBY01000091), *S. marcescens* Db11 (ftp.sanger.ac.uk/pub/pathogens/sm) and *S. proteamaculans* 568 (CP000826, CP000827). These sequence identities supported the inclusion of the *Serratia* isolate 4Rx13 in the *S. plymuthica* clade rather than the *S. odorifera* clade.

Volatile analysis

The profiles of headspace volatiles of four different species of the genus *Serratia* were analyzed by gas chromatography coupled to mass spectrometry (GC/MS) and compared with the emission of *S. plymuthica* 4Rx13 (Kai *et al.*, 2010; Fig. S1, Table S4). While growing on a complex medium (NBII), compounds were collected and analyzed in two growth phases (exponential growth phase 0–24 h and stationary growth phase 24–48 h) and analyzed. Altogether 98 compounds were detected from all investigated bacteria under these particular growth conditions. *Serratia proteamaculans* 568 and *S. marcescens* Db11 emitted 21 compounds in total, whereas 12 volatiles were detected and analyzed from *S. odorifera* DSM 4582 and *S. plymuthica* AS9. In contrast, the profile of *S. plymuthica* 4Rx13 contained the highest number of compounds (74 volatiles). Comparison of the profiles revealed that 50% of the compounds were different in the logarithmic and stationary growth phase of *S. proteamaculans* 568, *S. marcescens* Db11 and *S. plymuthica* AS9, indicating substantial metabolic shifts during growth. In contrast, 25% of the compounds of the volatile profiles of *S. odorifera* DSM 4582 and *S. plymuthica* 4Rx13 varied, suggesting less metabolic alterations. Similar results were obtained studying *E. coli, Salmonella enterica, Shigella flexneri, Candida tropicalis*, and *Xanthomonas campestris* pv. *vesicatoria* 85-10 volatile emissions (Bunge *et al.*, 2008; Kai *et al.*, 2010; Weise *et al.*, 2012).

Sixteen compounds were unequivocally identified in the four *Serratia* species: four alcohols, seven ketones, two sulfur compounds, one pyrazine, indole and sodorifen (Table 1). Interestingly, of these, only three were commonly emitted by all five species: 2-undecanone (#30), 2-tridecanone (#65), and 2-pentadecanone (#93). 2-Decanone (#22) was only released by *S. marcescens* Db11, indole (#32) only by *S. odorifera* DSM4582, and sodorifen (#40) only by *S. plymuthica* 4Rx13. Hydrocarbons, aliphatic alcohols and ketones are mostly produced during fatty acid biosynthesis (Schulz & Dickschat, 2007) and the search for the ketones 2-heptanone (#3), phenylacetone (#19), 2-decanone (#22), 2-undecanone (#30), 2-dodecanone (#47), 2-tridecanoncolour (#65), and 2-pentadecanone (#93) in the mVOC database

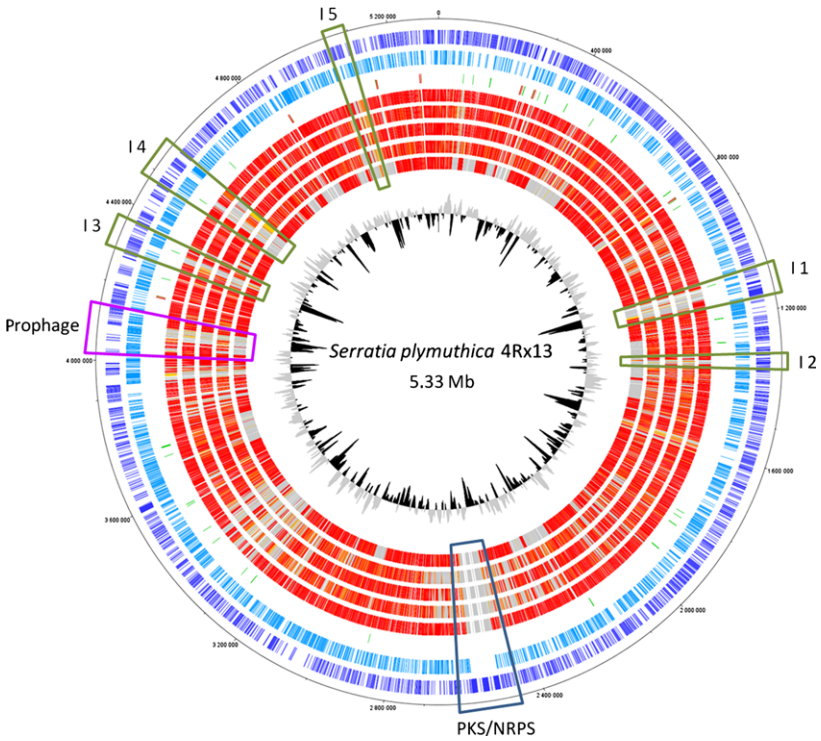

Fig. 2. Comparison of the chromosome of *Serratia plymuthica* 4Rx13 with other *Serratia* species. Genes encoded by the leading and the lagging strand (circle 1 and 2) of the chromosome of *S. plymuthica* 4Rx13 are marked in dark and light blue, respectively. rRNA gene clusters are shown in red and tRNAs in green (both 3rd circle). Genome comparison based on bidirectional BLASTS were done against *S. plymuthica* AS9 (4th circle, 44), *S. marcescens* DB11 (5th circle), *S. proteamaculans* 568 (6th circle), *S. odorifera* DSM4582 (7th circle) and *S. plymuthica* PRI-2C (8th circle). This results in the differentiation of core (red) and pane (gray) genome based on putative orthologous. The visualization was done with the DNA-Plotter tool (Carver *et al.*, 2009). The inner plot shows the G + C content. Green boxes are genomic islands (I 1–5) identified with ISLANDVIEWER, the pink box is the prophage region identified by PHAST and the petrol box presents the PKS/NRPS cluster.

Table 1. Identified volatiles emitted by *Serratia* species/isolates

Peak	Compound	RI	*S. proteamaculans* 568	*S. marcescens* Db11	*S. odorifera* DSM 4582	*S. plymuthica* AS9	*S. plymuthica* 4Rx13
1	3-methylbutanol		x	–	x	–	–
2	dimethyl disulfide	749	x	–	–	x	x
3	2-heptanone	899	x	x	x	–	–
4	2,3-dimethylpyrazine	932	x	x	–	–	x
6	dimethyl trisulfide	987	x	x	–	x	x
14	1-octanol	1076	–	x	–	x	–
18	2-phenylethanol	1125	x	x	x	–	x
19	phenylacetone	1142	x	x	x	–	x
22	2-decanone	1197	–	x	–	–	–
26	1-decanol	1276	x	x	x	x	–
30	**2-undecanone**	**1297**	**x**	**x**	**x**	**x**	**x**
32	indole	1314	–	–	x	–	–
40	sodorifen	1374	–	–	–	–	x
47	2-dodecanone	1400	x	x	–	–	x
65	**2-tridecanone**	**1501**	**x**	**x**	**x**	**x**	**x**
93	**2-pentadecanone**	**1705**	**x**	**x**	**x**	**x**	**x**

Volatiles in the headspaces of *S. proteamaculans* 568, *S. marcescens* Db11, *S. odorifera* DSM4582, *S. plymuthica* AS9 and *S. plymuthica* 4Rx13 were collected on SuperQ and subsequently analyzed by GC/MS. Volatiles were identified by retention indices, and co-injection of authentic reference substances and comparison of their mass spectra with those of compounds listed in the NIST147 library. Only unambiguously identified volatiles are listed in this Table.

RI, retention index; x, compound detected in headspace; –, compound not detected in headspace; compounds highlighted in gray are volatiles released by five *Serratia* species.

supported the widespread emission of ketones among bacteria (Effmert *et al.*, 2012; Lemfack *et al.*, 2013) (Table 2). Contrasting results were obtained regarding their biological functions: Bruce *et al.* (2004) discussed 2-undecanone (#30) as an antifungal compound, whereas Weise *et al.* (2012) observed slight growth promotion (15%) with *Rhizoctonia solani*.

2-Phenylethanol (#18) was emitted by four of five *Serratia* species. This alcohol is synthesized by the shikimate pathway via phenylalanine (Etschmann *et al.*, 2002) and is one of the most widespread volatile aromatic compounds released by microorganisms (Effmert *et al.*, 2012; Lemfack *et al.*, 2013). Bioassays with this alcohol revealed a concentration-dependent growth inhibition of *Arabidopsis thaliana* (Wenke *et al.*, 2012). Often 2-phenylethanol (#18) is released in combination with sulfur- and nitrogen-containing compounds (Schulz & Dickschat, 2007; Kai *et al.*, 2010). Therefore, it was not surprising that dimethyl disulfide (#2) and dimethyl trisulfide (#6) were present in the VOC mixtures of four of the five *Serratia* species analyzed here (Table 1). A concentration-dependent growth inhibition of *A. thaliana* as well as of the fungus *Fusarium culmorum* was also demonstrated with dimethyl disulfide (Kai *et al.*, 2009). In contrast, Meldau *et al.* (2013) showed that dimethyl disulfide promotes *Nicotiana attenuata* growth. Pyrazines, previously described as characteristic of *Serratia* species (Gallois & Grimont, 1985; *Serratia* spp., Bruce *et al.*, 2004; *S. marcescens*, Gu *et al.*, 2007) were released by *S. proteamaculans* 568, *S. marcescens* Db11 and *S. plymuthica* 4Rx13, but not by the other two species investigated here and by an *S. proteamaculans* isolate described by Ercolini *et al.* (2009) despite the fact that the latter produced a wealth of other volatile compounds. Pyrazines are emitted by

Table 2. The ketones emitted from *Serratia* sp. are released by other bacterial species

2-heptanone (#3)	*Phormidum* sp., *Pseudomonas* sp., *Rivularia* sp., *Streptomyces* sp., *Tolypothix distorta, Xanthomonas* sp.
phenylacetone (#19)	*Streptomyces* sp., *Klebsiella* sp.
2-decanone (#22)	*Phormidium* sp., *Rivularia* sp., *Tolyphotrix* sp.
2-undecanone (#30)	*Bacillus, Microbacterium* sp., *Pseudomonas* sp., *Serratia* sp., *Stenotrophomonas* sp., *Stigmatella* sp., *Streptomyces* sp., *Xanthomonas* sp.
2-dodecanone (#47)	*Arctic ice bacterium, Xanthomonas* sp.
2-tridecanone (#65)	*Phormidium* sp., *Plectonema* sp., *Pseudomonas* sp., *Rivularia* sp., *Tolypothrix* sp., *Xanthomonas* sp.
2-pentadecanone (#93)	*Arctic ice bacterium, Xanthomonas* sp.

Ketones were searched in mVOC database (Lemfack *et al.*, 2013).

many different bacterial species (Effmert *et al.*, 2012; Lemfack *et al.*, 2013). Therefore, pyrazines cannot further be used as a characteristic feature to identify the genus *Serratia*. Indole (#32), well known as a major compound of *E. coli* strains as well as *Enterobacter* and *Klebsiella* species (Schulz & Dickschat, 2007), was emitted by *S. odorifera* DSM4582. Application of indole on *A. thaliana* showed concentration-dependent growth effects (Blom *et al.*, 2011).

Sodorifen emission

The single emission of the bicyclic hydrocarbon sodorifen by *S. plymuthica* 4Rx13 is a remarkable feature (Table 1). Until now, no other bacterial species was known to emit this volatile compound, of which the biosynthesis and function is completely unknown. Sodorifen [1,2,4,5,6,7,8-heptamethyl-3-methylenebicyclo(3.2.1)oct-6-ene] is a unique polymethylated hydrocarbon where each carbon of the parent system is linked to at least three other carbons (von Reuss *et al.*, 2010). As sodorifen was emitted by *S. plymuthica* 4Rx13 (Kai *et al.*, 2010) and not by the related strain *S. plymuthica* AS9 (Table 1), we surveyed 15 strains to determine patterns of sodorifen production within members of the genus *Serratia* (Table 3). Sodorifen was not detected in *S. fonticola* V5706, *S. marcescens* Db11 and V11694, *S. odorifera* DSM4582, *S. proteamaculans* 568, or *S. rubidaea* V3095, but it could be found in three of seven *S. plymuthica* isolates. Interestingly, only isolates of branch II of section *S. plymuthica* released sodorifen, while no sodorifen was detected in the VOC profiles of the five species of branch I (Fig. 1, Table 3). Sodorifen was only produced by *S. plymuthica* strains that were isolated from plants, not by isolates that originated from humans or water. Furthermore, the sodorifen-producers of *S. plymuthica* were isolated from the rhizosphere of *Brassica napus* and the endorhiza of *Solanum tuberosum* that grew near Rostock (Germany), whereas the isolate AS9 obtained from *B. napus* growing in Sweden was a non-producer. Why the ability of *S. plymuthica* species to synthesize sodorifen synthesis evolved in fields near Rostock remains unknown. Detailed field or soil analyses are lacking but it is interesting to note that the fields had a long history of rape seed cultivation in common. Furthermore, the origin of sodorifen synthesis, including the presence of genes encoding the enzymes of the biosynthetic pathway (s), is presently unknown. Three scenarios may explain this phenotype: (1) either the *S. plymuthica* isolates from Rostock obtained new genes for the sodorifen biosynthesis via, for example, horizontal gene transfer to adapt to new environmental conditions/situations (Heuer & Smalla, 2012); (2) gene duplication and diversification occurred; or (3) the regulation of gene expression was altered, resulting in active biosynthetic enzymes.

Table 3. Distribution of sodorifen emission in the genus *Serratia*

Genus	Species	Isolate	Emission of sodorifen
Serratia	*plymuthica*	4Rx13	+
		HRO-C48	+
		3Re-4-18	+
		AS 9	–
		DSM 49	–
		DSM 4540	–
		DSM 30127	–
		PRI-2C	–
	liquefaciens	B 5319	–
	proteamaculans	568	–
	marcescens	V 11694	–
		Db 11	–
	odorifera	DSM 4582	–
	fonticola	V 5706	–
	rubidaea	V 3095	–

+, sodorifen was detected; –, sodorifen was not detected.

To investigate the hypothesis that the synthesis and emission of sodorifen by *S. plymuthica* 4Rx13 might result from the presence of unique genes in this isolate, the *S. plymuthica* 4Rx13 genome was compared with the non-producer genome sequences of *S. plymuthica* AS9 (Neupane *et al.*, 2013), *S. plymuthica* PRI-2C, *S. odorifera* DSM4582, *S. marcescens* Db11 and *S. proteamaculans* 568 by a bidirectional BLAST (Fig. 2). The phylogenetically closest isolate is *S. plymuthica* AS9. It differs from *S. plymuthica* 4Rx13 in only 435 ORFs, of which 244 ORFs were assigned a function (Tables S3 and S5). Compared with all non-sodorifen producers, the *S. plymuthica* 4Rx13 genome harbors 246 unique ORFs. These may present potential candidate genes in the sodorifen production. In total, 18 transcriptional regulators and 132 hypothetical proteins were identified within the unique ORFs of *S. plymuthica* 4Rx13. Additionally, several oxidoreductases and transporters were found. This pool of unique genes also includes one NRPS/PKS cluster (SOD_c22810 – SOD_c23030) that was described to be an oocydin A gene cluster (Matilla *et al.*, 2012). As a result, the involvement of this cluster in the sodorifen synthesis could be excluded. Furthermore, a one-phage region (genomic coordinates 4060600–4104861) and five genomic islands were identified with PHAST and ISLANDVIEWER, respectively (Fig. 2). To identify additional secondary metabolite clusters, the genome of 4Rx13 was analyzed with ANTISMASH (Table S5). This showed overlaps with the clusters of the other tools and three additional clusters, which did not harbor all of the unique ORFs. These clusters could be interesting candidates for the

synthesis of sodorifen. Most of the unique ORFs are located in genomic islands and phage regions (Table S5), they seem to be introduced through horizontal gene transfer. This can also recognized by their different GC content (51.7%) compared with all remaining ORFs (57.6%; Fig. 2).

Future work is necessary to elucidate the biological function(s) of sodorifen to unravel the underlying biosynthetic pathway and its origin.

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Authors' contribution

T.W. and A.T. contributed equally to the results presented.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Volatile profiles of several *Serratia* species.
Table S1. *Serratia* strains used.
Table S2. Genomic islands predicted with ISLANDVIEWER.
Table S3. Comparison of genome sequences of *Serratia* species/isolates.
Table S4. Volatiles emitted by *Serratia* species/isolates.
Table S5. Summary of all unique ORFs identified in *Serratia plymuthica* 4Rx13 with start and stop position, the best hit of BLASTP against Swiss-Prot and TrEMBL database (http://www.uniprot.org/downloads).