RESEARCH LETTER

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

Teresa Weise¹, Andrea Thürmer², Silja Brady², Marco Kai³, Rolf Daniel², Gerhard Gottschalk² & Birgit Piechulla¹

¹Institute of Biological Sciences, University of Rostock, Rostock, Germany; ²Department of Genomic and Applied Microbiology & Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, Georg-August University Göttingen, Göttingen, Germany; and ³Max-Planck-Institute for Chemical Ecology, Jena, Germany

Correspondence: Birgit Piechulla, Institute of Biological Sciences, University of Rostock, Albert-Einstein-Str. 3, 18059 Rostock, Germany. Tel.: + 49 (0)381 4986130; fax: + 49 (0)381 4986132; e-mail: birgit.piechulla@uni-rostock.de

Received 27 September 2013; revised 3 December 2013; accepted 10 December 2013. Final version published online 7 January 2014.

DOI: 10.1111/1574-6968.12359

Editor: Michael Galperin

Keywords
Serratia plymuthica; Serratia odorifera; 16S rRNA gene phylogenetic tree; sodorifen emission; volatile emission profiles; volatile organic compounds.

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-Marín 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-amin-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 (Fe²⁺) (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 oocycin A acting against oomycetes (Matilla et al., 2012) and althiomyacin, 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.,...
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\textsuperscript{TM} complete DNA purification kit, Epicentre, Madison, WI). 16S rRNA genes were amplified using the forward primer 16S-08_for 5′-AGAGTTTGTATCCTGGC-3′, and the reverse primer 16S-1504_rev 5′-TACCTTGTGACTTAC-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\textsuperscript{TM} 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 program.

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 croatus*, *Carriobacterium diversigen*, *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 synthetic biology and its ecological relevance is presently unknown, this novel and highly remarkable structure raised the question of its synthetic origin of the species. 16S rRNA gene sequences and genome comparisons 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.
Sodorifen released by Serratia plymuthica 4Rx13

asssembler 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 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/home.php), and potential secondary metabolite clusters were analyzed with ANTSMASS (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 strain 50_2A. The 16S rRNA gene analysis also placed 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 (ABDY01000000-ABDY01000091), 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-tridecanone (#65), and 2-pentadecanone (#93) in the mVOC database...
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 1.** Identified volatiles emitted by Serratia species/isolates

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>S. proteamaculans 568</th>
<th>S. marcescens Db11</th>
<th>S. odorifera DSM 4582</th>
<th>S. plymuthica A59</th>
<th>S. plymuthica 4Rx13</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-methylbutanol</td>
<td>x</td>
<td>–</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>dimethyl disulfide</td>
<td>749</td>
<td>x</td>
<td>–</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td>2-heptanone</td>
<td>899</td>
<td>x</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>2,3-dimethylpyrazine</td>
<td>932</td>
<td>x</td>
<td>x</td>
<td>–</td>
<td>x</td>
</tr>
<tr>
<td>6</td>
<td>dimethyl trisulfide</td>
<td>987</td>
<td>x</td>
<td>x</td>
<td>–</td>
<td>x</td>
</tr>
<tr>
<td>14</td>
<td>1-octanol</td>
<td>1076</td>
<td>–</td>
<td>x</td>
<td>–</td>
<td>x</td>
</tr>
<tr>
<td>18</td>
<td>2-phenylethanol</td>
<td>1125</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>19</td>
<td>phenylacetone</td>
<td>1142</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>–</td>
</tr>
<tr>
<td>22</td>
<td>2-decane</td>
<td>1197</td>
<td>–</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>26</td>
<td>1-decanol</td>
<td>1276</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>30</td>
<td>2-undecanone</td>
<td>1297</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>32</td>
<td>indole</td>
<td>1314</td>
<td>–</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>40</td>
<td>sodorifen</td>
<td>1374</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>x</td>
</tr>
<tr>
<td>47</td>
<td>2-dodecanone</td>
<td>1400</td>
<td>x</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>65</td>
<td>2-tridecanone</td>
<td>1501</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>93</td>
<td>2-pentadecanone</td>
<td>1705</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

Volatiles in the headspaces of S. proteamaculans 568, S. marcescens Db11, S. odorifera DSM4582, S. plymuthica A59 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.

---

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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2-decanone (#22)</td>
<td>Phormidium sp., Rivularia sp., Tolypothix sp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-undecanone (#30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-dodecanone (#47)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-tridecanone (#65)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-pentadecanone (#93)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

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-phase region (genomic coordinates 4060600–4104861) and five genomic islands were identified with PHAST and ISLAND- VIEWER, respectively (Fig. 2). To identify additional secondary metabolite clusters, the genome of 4Rx13 was analyzed with ANTIMASH (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

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Isolate</th>
<th>Emission of sodorifen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serratia</td>
<td>plymuthica</td>
<td>4Rx13</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HRO-C48</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3Re-4-18</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS 9</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DSM 49</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DSM 4540</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DSM 30127</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PRI-2C</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>proteamaculans</td>
<td>B 5319</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>568</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>marcescens</td>
<td>V 11694</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Db 11</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>odorifera</td>
<td>DSM 4582</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>fonticola</td>
<td>V 5706</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>rubidaea</td>
<td>V 3095</td>
<td>–</td>
</tr>
</tbody>
</table>

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

Acknowledgements

The authors thank A. Podbielsky and B. Kreikemeyer (Medical Faculty, University of Rostock, Germany) for the isolates (S. marcescens V11694, S. rubidaea. V3095, S. fonticola V5706, S. liquefaciens B5319), S. Neupane and S. Alstäm (Uppsala BioCenter, Sweden) for the S. plymuthica AS9 strain, J. Ewbank (Centre d’Immunologie de Marseille Luminy, France) for the S. marcescens Db11, D.v.d. Lelie (Brookhaven National Laboratory, Upton) for the S. proteamaculans 568, G. Berg (TU, Graz, Austria) for S. plymuthica 4Rx13, 3Re-4-18 and HRO-C48, and Paolina Garbeva (NIOO, Wageningen, the Netherlands) for the PRI-2C isolate. We thank W. Francke (Hamburg, Germany) for critical reading of the manuscript and reference compounds. The work of B.P. was financially supported by the DFG. Provision of the project by the Goettingen Genomics Laboratory is acknowledged.

Authors’ contribution

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

References


*Procaryotes* 6: 219–244.

Grimont PAD, Grimont F & Dulong de Rosnay HLC (1977) The 

Grimont PAD, Grimont F, Richard C, Davis BR, Steigerwalt 
AG & Brenner DJ (1978) Desoxyribonucleic acid relatedness 
between *Serratia plymuthica* and other *Serratia* species with a 
description of *Serratia odorifera* sp. (Type strain: ICPB 3995). 

Gu Y-Q, Ming-He M, Zhou J-P, Zou C-S & Zhang K-Q 
nematicidal volatiles from soil bacteria. *Soil Biol Biochem* 39: 
2567–2575.

recognizing protein-coding genes in bacterial and archaeal 

Heuer H & Smalla K (2012) Plasmids foster diversification and 
adaptation of bacterial populations in soil. *FEMS Microbiol 
Rev* 36: 1083–1104.

*Mammalian Protein Metabolism*, (Munro LN, Ed), pp. 

(2009) Bacterial volatiles and their action potential. *Appl 

Kai M, Crespo E, Cristescu SM, Harren FJM, Francke W & 
emission and biological impact of volatile compounds on 

*Serratia* as beneficial rhizobacteria of oilseed rape. *Microbiol 

Lemfack MC, Nickel J, Dunkel M, Preissner R & Piechulla B 

software environment for sequence data. *Nucleic Acids Res* 
32: 1363–1371.

Mahlen SD (2011) *Serratia* infections: from military experiments 

Comparative genomics of *Serratia* spp.: two paths towards 

Martinec T & Kocur M (1961) A taxonomic study of the 
members of the genus *Serratia*. *Int Bull Bacteriol Nomencl 
Taxon* 11: 73–78.

Matilla MA, Stöckmann H, Leeper FJ & Salmond GPC (2012) 
Identification of oocydin A biosynthetic gene clusters. *J Biol 

Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski 
Rapid identification, annotation and analysis of secondary 
metabolite biosynthesis gene clusters. *Nucleic Acids Res* 39: 

Meldau DG, Meldau S, Hoang LH, Underberg S, Wünsche H 
& Baldwin IT (2013) Dimethyl disulfide produced by the 
native *Bacillus* sp. B55 promotes *Nicotiana attenuata* growth by enhancing sulfur nutrition. *Plant Cell* 25: 
2731–2747.

Müller H, Westendorf C, Leitner E, Chernin L, Riedel K, 
Schmidt S, Eberl L & Berg G (2008) Quorum sensing 
effects in the antagonistic rhizosphere bacterium 
*Serratia plymuthica* HR8-C48. *FEMS Microbiol Ecol* 67: 
468–478.

Muyzer G, de Waal EC & Uitterlinden AG (1993) Profiling of 
complex microbial populations by denaturing gradient gel 
electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ 

Nadkarni MA, Martin FE, Jacques NA & Hunter N (2002) 
Determination of bacterial load by real-time PCR using a 
broad-range (universal) probe and primers set. *Microbiology* 148: 257–266.

Neupane S, Högborg N, Alström S et al. (2012) Complete 
genome sequence of the rapeseed plant-growth 
promoting *Serratia plymuthica* AS9. *Stand Genomic Sci* 6: 
54–62.

Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, 
sequence visualization and annotation. *Bioinformatics* 16: 
944–945.

a new method for reconstructing phylogenetic trees. *Mol Biol 

Schulz S & Dickschat JS (2007) Bacterial volatiles: the smell of 

Phosphate availability regulates biosynthesis of two 
antibiotics, prodigiosin and carbapenem, in *Serratia* via 


obtained by a combination of existing methods. *In Silico 

Octamethylbicyclo[3.2.1]octadienes from *Serratia odorifera*. 
*Angew Chem* 122: 2053–2054, 2010; *Angew Chem Int Ed* 49: 

Weise T, Kai M, Gummesson A et al. (2012) Volatile organic 
compounds produced by the phytopathogenic bacterium 
*Xanthomonas campestris* pv. *vesicatoria* 85-10. *Beilstein J Org 
Chem* 8: 579–596.

Weise T, Kai M, Gummesson A et al. (2012) Volatile organic 
compounds produced by the phytopathogenic bacterium 
*Xanthomonas campestris* pv. *vesicatoria* 85-10. *Beilstein J Org 
Chem* 8: 579–596.
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).