Genome Sequences of Serratia Strains Revealed Common Genes in Both Serratomolides Gene Clusters

Catarina Marques-Pereira †, Diogo Neves Proença * and Paula V. Morais ‡

Department of Life Sciences, Centre for Mechanical Engineering, Materials and Processes, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal; catarina.103@gmail.com (C.M.-P.); pvmorais@uc.pt (P.V.M.)

* Correspondence: diogo.proenca@uc.pt; Tel.: +351-239240798
† Current address: Center for Neuroscience and Cell Biology & Center for Innovative Biomedicine and Biotechnology, University of Coimbra, 3004-504 Coimbra, Portugal.

Received: 31 October 2020; Accepted: 17 December 2020; Published: 20 December 2020

Simple Summary: Biosurfactants are amphiphilic molecules produced by microorganisms with a hydrophilic and a hydrophobic group, able to reduce surface tension. These molecules are largely used in the environmental, food, pharmaceutical, medical, and cleaning industries, among others. Serratia strains are ubiquitous microorganisms with the ability to produce biosurfactants, such as serrawettins. These extracellular lipopeptides are described as biocides against many bacteria and fungi. This work used comparative genomics to determine the distribution and organization of the serrawettins W1 and W2 biosynthetic gene clusters in all the 84 publicly available genomes of the Serratia genus. Here, the serrawettin W1 gene clusters’ organization is reported for the first time. The serrawettin W1 biosynthetic gene swrW and serrawettin W2 biosynthetic gene swrA were present in 17 and 11 Serratia genomes, respectively. The same genes in the biosynthetic clusters frame the swrW and swrA biosynthetic genes. This work identified four genes common to all serrawettin gene clusters, highlighting their key potential in the serrawettins biosynthetic process.

Abstract: Serratia strains are ubiquitous microorganisms with the ability to produce serratomolides, such as serrawettins. These extracellular lipopeptides are described as biocides against many bacteria and fungi and may have a nematicidal activity against phytopathogenic nematodes. Serrawettins W1 and W2 from different strains have different structures that might be correlated with distinct genomic organizations. This work used comparative genomics to determine the distribution and the organization of the serrawettins biosynthetic gene clusters in all the 84 publicly available genomes of the Serratia genus. The serrawettin W1 and W2 gene clusters’ organization was established using antiSMASH software and compared with single and short data previously described for YD25 T Serratia. Here, the serrawettin W1 gene clusters’ organization is reported for the first time. The serrawettin W1 biosynthetic gene swrW was present in 17 Serratia genomes. Eighty different coding sequence (CDS) were assigned to the W1 gene cluster, 13 being common to all clusters. The serrawettin W2 swrA gene was present in 11 Serratia genomes. The W2 gene clusters included 68 CDS with 24 present in all the clusters. The genomic analysis showed the swrA gene constitutes five modules, four with three domains and one with four domains, while the swrW gene constitutes one module with four domains. This work identified four genes common to all serrawettin gene clusters, highlighting their essential potential in the serrawettins biosynthetic process.

Keywords: Serratia; genome; serrawettin; biosynthetic gene cluster; serratomolides; swrW gene; swrA gene
1. Introduction

Surfactants are amphiphilic molecules with a hydrophilic and a hydrophobic group, able to reduce surface tension. These molecules are largely used in the environmental, food, pharmaceutical, medical, and cleaning industries, among others [1–3]. Biosurfactants are secondary metabolites produced by bacteria, yeast, or fungi, capable of reducing the surface tension of extracellular media [4]. They can have a variety of structures, mainly divided into six major groups, namely, glycolipids, lipopolysaccharides, lipopeptides and phospholipids, and hydroxylated and cross-linked fatty acids [5]. When compared with synthetic surfactants, biosurfactants have higher biodegradability, lower toxicity, and higher activity at extreme conditions, such as high temperatures, pH, and salinity [6].

Bacterial species of the genus *Serratia* belonging to the family *Enterobacteriaceae* are Gram-negative, facultative anaerobic, and rod-shaped bacteria [7,8]. They have been isolated from different environments, such as water, soil, plants, insects, and vertebrates [9]. Surfaces in these habitats have a variety of characteristics and can be hydrophobic, hydrophilic, fractal, smooth, and axenic, among others. To colonize them, bacteria from the genus *Serratia* produce biosurfactants (extracellular lipopeptides) as the serratomolides serrawettin W1 [10], serrawettin W2 [4], and serrawettin W3 [11]. Mutants for the serratomolides biosynthetic genes failed to form colonies on nutritive agar plates and these lipopeptides were suggested as promoters of a new type of spreading growth [4].

Both the serrawettin W1 and W2 biosynthetic genes (*swrW* and *swrA* genes) belong to a Non-Ribosomal Peptide Synthetase (NRPS) gene cluster [12,13]. Condensation, adenylation, thiolation, and thioesterase domains are detectable in both serrawettin gene clusters. Along with other proteins, such as PPTase or acyl carrier proteins (ACP) [12,14], serrawettin W1 and W2 biosynthetic proteins are able to synthesize serrawettin W1 and W2 lipopeptides. Serrawettin W1 has a symmetric structure composed of two serine amino acids and two fatty acids (3-hydroxydecanoic) [4] while serrawettin W2 is a cyclic peptide containing five amino acids. This lipopeptide promotes flagellum spreading growth, contributing specifically to surface bacterial translocation [4], and was able to demonstrate antimicrobial activity against many bacteria and fungi and an antitumoral activity against Hela cells [13]. The *Serratia* strains producing serrawettin W1 showed the highest broad-spectrum antimicrobial activity against clinical, food, and environmental bacterial pathogens compared with the *Serratia* strains producing serrawettin W2 [15]. A strain of *S. marcescens* showed the capacity to inhibit an endophytic fungus due to its production of compounds, namely, serrawettins [16].

Due to the limited information on this topic, in this study, we performed a comparative genomic analysis to determine the distribution and the organization of the serrawettins biosynthetic gene clusters in all the 84 publicly available genomes of the *Serratia* genus. Here, we report a deep analysis of the serrawettin W1 and W2 gene clusters and establish their organization using antiSMASH software. In particular, for the first time, we show the serrawettin W1 gene clusters’ organization. These analyses showed the presence of four genes common to all the serrawettin gene clusters, highlighting their key potential in the serrawettins biosynthetic process.

2. Materials and Methods

2.1. Bacterial Strains, Data Collection, and Genomes

Eighty-four *Serratia* genomes available on NCBI, representing all the publicly available genomes, were included in this study (Table S1), obtained from 49 different hosts and isolated from 29 countries [13,17–49].

Serrawettin W1 and W2 biosynthetic gene cluster prediction was performed in all the 84 complete and draft *Serratia* genomes publicly available, using the web platform antiSMASH 3.0 [50]. This input dataset includes strains belonging to *S. marcescens*, *S. liquefaciens*, *S. grimesii*, *S. nematodiphila*, *S. plymuthica*, and *S. ureilytica*, as well as strains not characterized to the species level. AntiSMASH analysis output was examined to identify the serrawettins biosynthetic genes clusters. Therefore, to find the *swrW* and *swrA* genes, BLAST analysis was performed for all protein sequences, codified by
each gene of the NRPS predicting metabolite clusters, using the NCBI [51] database. Cluster boundaries were predicted with the CASSIS algorithm specified for the NRPS domains [50].

2.2. Phylogenetic Analysis

The 16S rRNA sequences of the 47 Serratia strains, which showed the presence of serrawettins genes in their genomes (Table 1), were selected for phylogenetic analysis. These sequences were compared with the sequences available in the EMBL/GenBank database using the BLASTN network services [51], and with sequences available at the Eztaxon-e server (http://eztaxon-e.ezbiocloud.net/) [52]. Sequences were aligned within the SINA alignment service [53]. Sequences were included in the 16S rRNA-based Living Tree Project (LTP, release 115) database (http://www.arb-silva.de/projects/living-tree/) by parsimony implemented in the ARB software package version 5.5 [54]. Phylogenetic dendrograms of this study strains and closest reference sequences were constructed using the Neighbor-Joining and Randomized Axelerated Maximum Likelihood (RAxML) method with the GTRGAMMA model [55] included in the ARB software [54].

Table 1. Serratia strains with the swrW and swrA genes identified through antiSMASH software. AntiSMASH software was used in 84 Serratia genomes (see Table S1). X represents the presence of genes swrW and swrA in seventeen and eleven genomes, respectively.

| Bacterial Strain                      | Accession Number | swrW | swrA |
|---------------------------------------|------------------|------|------|
| Serratia sp. AS12                     | CP002774.1       | X    |      |
| Serratia sp. AS13                     | CP002775.1       | X    |      |
| Serratia sp. FS14                     | CP005927.1       | X    |      |
| Serratia sp. SCBI                     | CP03424.1        |      | X    |
| Serratia sp. YD25                     | CP016948.1       | X    |      |
| Serratia sp. SSIH1                    | CP026383.1       | X    |      |
| Serratia sp. PWN146                   | LT575490.1       | X    |      |
| Serratia marcescens strain UMH8       | CP018927.1       |      | X    |
| Serratia marcescens strain IOMTU 115  | AB894481.1       | X    |      |
| Serratia marcescens subsp. marcescens ATCC 13880 | JMPQ01000033.1 | X    |      |
| Serratia marcescens strain CDC_813-60 DP21 | JOMV01000041.1 | X    |      |
| Serratia nematodiphila DZ0503SB1 strain DSM 21420 | JPUX00000000.1 | X    |      |
| Serratia marcescens VGH107             | AORJ0000000.1    | X    |      |
| Serratia marcescens EGD-HP20           | AVSR0000000.1    | X    |      |
| Serratia marcescens WW4                | CP003999.1       | X    |      |
| Serratia marcescens BIDMC 31          | JMJZ01000006.1   | X    |      |
| Serratia strain TEL_NODE_13           | LEDG0100018.1    | X    |      |
| Serratia plymuthica NBRC 102599T      | BCTU00000000.1   | X    |      |
| Serratia grimesii isolate BXF1         | LT883155.1       | X    |      |
| Serratia grimesii strain A2            | JGP00000000.1    | X    |      |
| Serratia plymuthica AS9                | CP002773.1       | X    |      |
| Serratia marcescens SM39               | AP013063.1       | X    |      |
| Serratia marcescens SmUNAM836          | CP012685.1       | X    |      |
| Serratia marcescens BIDMC 44           | JAPD01000005.1   | X    |      |
| Serratia ureilytica Lr5/4 LG59         | JSFB0100001.1    | X    |      |
| Serratia marcescens RSC-14             | CP012639.1       | X    |      |
| Serratia marcescens subsp. marcescens AH0650_Sm1 AG2 | LFJS01000014.1 | X    |      |
| Serratia marcescens subsp. marcescens Dh11 | HG326223.1      | X    |      |

2.3. Serrawettins Biosynthetic Gene Clusters Analysis

The NRPS gene cluster, where both the serrawettin W1 and W2 biosynthetic genes belong, was analyzed here by using antiSMASH software [50]. The reconstruction of the domains, modules, and structures of the serrawettins was performed by using Phyre2 tridimensional prediction [56], using the serrawettin W1 biosynthetic protein from Serratia sp. AS13, and W2 from Serratia sp. PWN146. PubChem 2D was used to explore the chemical information of the serrawettins using the bacterial models mentioned above.

The concatenated amino acid sequences of each serrawettin biosynthetic gene cluster were organized by protein identification in the same order to allow a cluster alignment with ClustalW
in MEGAX software [57]. The serrawettin W1 biosynthetic proteins from 17 *Serratia* genomes and serrawettin W2 biosynthetic proteins from 11 *Serratia* genomes were separately aligned. The evolutionary relationship between the clusters and between the serrawettin biosynthetic protein sequences were established using the Neighbor-Joining method, Poisson model [58], in MEGAX software [57]. The aligned and organized cluster genes were represented in an evolutionary tree according to the size, direction, and accession numbers identified with the NCBI BLAST database [51]. Functions of the core proteins in all clusters were searched on UniProt [59].

3. Results

3.1. Bacterial Phylogeny and Comparative Genomics of *Serratia* spp.

All the 84 *Serratia* strains selected for this study had their genome publicly available at NCBI. According to the Neighbor-Joining and Maximum-Likelihood phylogenetic trees based on 16S rRNA gene sequences, 14 strains belong to *S. marcescens*, two strains belong to *S. plymuthica*, two strains belong to *S. grimesii*, one strain belongs to *S. liquefaciens*, one strain belongs to *S. nematodiphila*, one strain belongs to *S. ureilytica*, and 63 strains could not be assigned to species level due to a similarity lower than 97% (Figure 1). Genomes of the *Serratia* strains have a size from 5.0 Mbp to 7.7 Mbp and the G + C content varies from 45.9 to 60.1 mol%.

![Figure 1](https://example.com/serratia_phylogeny.png)

**Figure 1.** A phylogenetic dendrogram based on a comparison of the 16S rRNA gene sequence of the *Serratia* strains used in this study and the type of strains. The tree was created using the Neighbor-Joining method in ARB software. The numbers on the tree indicate the percentages of bootstrap sampling, derived from 1000 replications; values below 50% are not shown. In blue are shown the strains that showed the presence of the serrawettin W1 biosynthetic gene cluster and in green are shown the *Serratia* strains that showed the presence of the serrawettin W2 biosynthetic gene cluster. The type species *Escherichia coli* DSM 30083T was used as the outgroup. Scale bar, 1 inferred nucleotide substitution per 100 nucleotides.

Bioinformatic analysis through antiSMASH software showed that the serrawettin W1 biosynthetic gene was present in 17 *Serratia* genomes, strains ATCC 13880, CDC_813-60 DP21, UMH8, IOMTU 115, DSM 21420, VGH107, EGD-HP20, WW4, FS14, BIDMC81, TEL NODE_13, NBRC 102599T, BXF1, A2, AS13, AS9, and AS12 (Table 1 and Table S2). The serrawettin W2 biosynthetic gene was present in 11
Serratia strains, PWN146, SSNIH1, SM39, SmUNAM836, BIDMC 44, Lr5/4 LG59, RSC-14, AH0650_Sm1 AG2, Db11, SCBI, and YD25T (Table 1 and Table S3).

3.2. Serrawettin W1 Biosynthetic Gene Clusters

All serrawettin W1 biosynthetic gene clusters were identified as NRPS clusters. Moreover, the bioinformatic analysis predicted an architecture including the domains condensation (C), adenylation (A), thiolation (T), and thioesterase (TE) in all the serrawettin W1 biosynthetic genes (Figure 2).

![Figure 2](image-url). Serrawettin W1: from the biosynthetic gene cluster to the structure of serrawettin W1. Genetic organization of the genome of Serratia strain AS13 by antiSMASH analysis, Phyre2 tridimensional prediction of serrawettin W1 biosynthetic protein, and PubChem 2D structure of serrawettin W1. (a) Serrawettin W1 biosynthetic gene cluster with identification of the srwW core gene; (b) srwW gene organization: C, condensation domain; A, adenylation domain; T, thiolation domain; and TE, thioesterase domain; (c) biosynthetic protein with the condensation, adenylation, thiolation, and thioesterase domains; (d) serrawettin W1 2D structure.

To confirm the identification of the *srwW* gene revealed by antiSMASH, each *srwW* was queried to NCBI BLASTP, in order to find the closest relative and determine the identity percentage (Table 2 and Table S2). The protein from the serrawettin W1 biosynthetic gene (*srwW*) showed an identity percentage that ranges from 77.79% to 100% as the closest identification by using BLASTP (Table 2).
Table 2. The serrawettin W1 gene (swrW) of each strain in this study and the accession numbers and identification of the swrW gene’s closest relatives using BLASTP.

| Bacterial Strain                      | swrW Closest Relative Genes                           | Accession Number | Identity Percentage |
|---------------------------------------|------------------------------------------------------|------------------|---------------------|
| *Serratia marcescens* EGD-HP20        | non-ribosomal peptide synthetase                     | ERH70695.1       | 99.52               |
| *Serratia marcescens* WW4             | serrawettin W1 synthetase                            | AGE20181.1       | 100                 |
| *Serratia* sp. FS14                   | putative serrawettin W1 synthetase                   | AIA46701.1       | 100                 |
| *Serratia marcescens* VGH107          | amino acid adenylation protein                       | EMF04443.1       | 99.12               |
| *Serratia* nematodiphila DZ0503SBS1 strain DSM 21420 | non-ribosomal peptide synthetase                     | KFF87803.1       | 99.31               |
| *Serratia marcescens* strain IOMTU 115 | putative serrawettin W1 synthetase                   | BAO21138.1       | 99.92               |
| *Serratia marcescens* strain UMH8     | non-ribosomal peptide synthetase                     | ASM18665.1       | 99.01               |
| *Serratia marcescens* subsp. marcescens ATCC 13880 | amino acid adenylation domain-containing protein       | KFD14984.1       | 98.57               |
| *Serratia marcescens* strain CDC_813-60 DP21 | non-ribosomal peptide synthetase                     | KFL05097.1       | 98.63               |
| *Serratia marcescens* BIDMC 81        | non-ribosomal peptide synthetase                     | EZQ62923.1       | 95.12               |
| *Serratia* strain TEL NODE_13         | non-ribosomal peptide synthetase                     | KLE36484.1       | 95.05               |
| *Serratia plymuthica* NBRC 102599T    | non-ribosomal peptide synthetase                     | WP_063202307.1   | 81.71               |
| *Serratia* sp. AS13                   | non-ribosomal peptide synthetase                     | AEG30284.1       | 81.48               |
| *Serratia plymuthica* AS9             | non-ribosomal peptide synthetase                     | AEF47625.1       | 81.48               |
| *Serratia* sp. AS12                   | non-ribosomal peptide synthetase                     | WP_013814722.1   | 81.48               |
| *Serratia grimesii* isolate BXF1      | amino acid adenylation domain-containing protein       | SMZ58711.1       | 77.79               |
| *Serratia grimesii* strain A2         | non-ribosomal peptide synthetase                     | KFB89923.1       | 78.17               |

Eighty different genes from the serrawettin W1 biosynthetic gene cluster were identified. Fifteen genes are common to all 17 gene clusters (Figure 3 and Table S4), such as genes encoding for murein hydrolase effector protein LrgB and murein hydrolase regulator LrgA, both with hydrolase activity; LysR regulatory protein with DNA-binding transcription factor activity; a sodium-hydrogen antiporter and xanthine-uracil-vitamin C permease, both with transmembrane transport activity; glyoxalase–bleomycin resistance protein and glutathione S-transferase domain protein, both with a dioxygenase activity; 3-oxoacyl-(acyl-carrier-protein) reductase; single-stranded DNA-binding protein; exonuclease ABC subunit A; maltose O-acetyltransferase; and aromatic amino acid aminotransferase. Not considering strain NRBC 102599T, an additional seven genes were found to be common to all strains in the serrawettin W1 biosynthetic gene cluster (Figure 3). Moreover, 15 genes are exclusive to the *S. plymuthica* NBRC 102599T biosynthetic gene cluster (Figure 3).
Figure 3. Serrawettin W1 gene cluster analysis. The phylogenetic relationship was established with Mega X software by the Neighbor-Joining method on aligned serrawettin W1 gene clusters of *Serratia* strains ATCC 13880, CDC-S83-60 DP21, UMH8, IOMTU 115, DSM 21420, VGH107, EGD-HP20, WW4, FS14, BIDMC81, TEL NODE_13, NBRC 102599, BXF1, A2, AS13, AS9, and AS12. (a) Phylogenetic tree based on protein sequences of the serrawettin W1 biosynthetic gene cluster on an established genetic organization. (b) Serrawettin W1 biosynthetic gene clusters based on natural genetic organization. (c) Comparison of the phylogenetic tree based on protein sequences of the serrawettin W1 biosynthetic gene clusters (left) with the phylogenetic tree of the serrawettin W1 biosynthesis protein (right). The scale bar of 0.050 infers the nucleotide substitutions per 100 nucleotides.
The relationship between strains established based on the analysis of the concatenated genes of the W1 biosynthetic gene cluster defined the same clusters as the relationships defined based on the swrW gene analysis, except for *S. plymuthica* NBRC 102599\textsuperscript{T}, which is discordant. The position of *S. plymuthica* NBRC 102599\textsuperscript{T} in W1 phylogenetic tree highlights the different gene composition of the W1 biosynthetic cluster of the strain. On the other hand, in the swrW phylogenetic tree, *S. plymuthica* NBRC 102599\textsuperscript{T} forms a sister group with *Serratia* strains AS13, AS9, and AS12 (Figure 3).

In the serrawettin W1 biosynthetic gene cluster, software prediction identified four genes involved in PKS-NRPS (Polyketide Synthases Non-Ribosomal Peptide Synthetases), encoding for enoylreductase quinone oxidoreductase (only present in seven strains), ketoreductase 3-oxoacyl-(acyl-carrier-protein) reductase (present in all strains), enoylreductase dehydrogenase (absent in two strains), and aromatic amino acid aminotransferase (present in all strains) (Table 3 and Table S4, Figure 3).

### Table 3. The PKS gene accession numbers from the swrW biosynthetic gene clusters predicted by antiSMASH software.

| Bacterial Strain | Enoylreductase Quinone Oxidoreductase | 3-Oxacyl-(Acyl-Carrier-Protein) Reductase | Enoylreductase Dehydrogenase | Aromatic Amino Acid Aminotransferase |
|------------------|--------------------------------------|------------------------------------------|-------------------------------|-------------------------------------|
| *Serratia marcescens* EGD-HP20 | ERH70706.1 | ERH70710.1 | ERH70714.1 |
| *Serratia marcescens* WW4 | AGE20192.1 | AGE20197.1 | AGE20201.1 |
| *Serratia* sp. FS14 | AIA46690.1 | AIA46685.1 | AIA46681.1 |
| *Serratia marcescens* VGH107 | EMF04432.1 | EMF04427.1 | EMF04423.1 |
| *Serratia nematophilus* DZ0505BS1 strain DSM 21420 | KFF87792.1 | KFF87787.1 | KFF87783.1 |
| *Serratia marcescens* strain IOMTU 115 | BAO21148.1 | BAO21153.1 | BAO21155.1 |
| *Serratia marcescens* strain UMH8 | ASM18675.1 | ASM18681.0 | ASM18684.1 |
| *Serratia marcescens* subsp. *Marcescens* ATCC 13880 | KFD14974.1 | KFD14969.1 | KFD14965.1 |
| *Serratia marcescens* strain CDC_813-60 DP21 | KFL04091.1 | KFL03204.1 | KFL04717.1 |
| *Serratia marcescens* BDMC 81 | EZQ62938.1 | EZQ62913.1 | EZQ62903.1 |
| *Serratia marcescens* strain TEL NODE_13 | KLE36470.1 | KLE36494.1 | KLE36503.1 |
| *Serratia plymuthica* NBRC 102599\textsuperscript{T} | WP_0063202297.1 | WP_006328332.1 | WP_006328339.1 |
| *Serratia* sp. AS13 | AEG30270.1 | AEG30284.1 | AEG30297.1 |
| *Serratia plymuthica* AS9 | AEF47611.1 | AEF47635.1 | AEF47638.1 |
| *Serratia* sp. AS12 | WP_013814732.1 | WP_013814734.1 | WP_013814736.1 |
| *Serratia grimesii* isolate BXF1 | SMZ58698.1 | SMZ58721.1 | SMZ58727.1 |
| *Serratia grimesii* strain A2 | KFB89913.1 | KFB89904.1 |

### 3.3. Serrawettin W2 Biosynthetic Gene Clusters

Every serrawettin W2 biosynthetic gene (swrA) showed an architecture composed of five modules, each with a condensation (C1, C2, C3, C4, and C5), adenylation (A1, A2, A3, A4, and A5), and thiolation (T1, T2, T3, T4, and T5) domain. Module 5 has an additional thioesterase (TE) domain. This organization is shared by all the serrawettin W2 biosynthetic genes (Figure 4).
Twenty-four genes were present in all the strains’ gene clusters (Figure 5), namely, the biology using BLASTX (Table 4). The protein coded by the biosynthetic genes of serrawettin W2 shown above, queried to NCBI BLASTX, in order to find the closest relative and determine the identity percentage (Tables 4 and S3). The protein coded by the biosynthetic genes of serrawettin W2 showed an identity percentage that ranges from 76.38% to 99.4% as the closest identification by using BLASTX (Table 4).

Table 4. The serrawettin W2 gene (swrA) of each strain in this study and the accession numbers and identification of the swrA gene’s closest relatives using BLASTX.

| Bacterial Strain | Serratia Closest Relative Genes | Accession Number | Identity Percentage |
|------------------|---------------------------------|------------------|---------------------|
| Serratia marcescens SM39 | Serratia marcescens SM39 DNA, complete genome | BAC05825.1 | 76.70 |
| Serratia marcescens SmUNAM836 | Serratia marcescens strain SmUNAM836, complete genome | ALE98111.1 | 76.70 |
| Serratia sp. SSNIH1 | Serratia sp. SSNIH1 chromosome, complete genome | AUY16873.1 | 76.70 |
| Serratia sp. PWN146 | Serratia marcescens isolate PWN146, assembly genome assembly, chromosome | SAY45247.1 | 77.02 |
| Serratia ureilytica Lr5/4 LG59 | Serratia marcescens strain UM17, complete genome | KKO55915.1 | 76.38 |
| Serratia marcescens RSC-14 | Serratia marcescens strain RSC-14, complete genome | ALD45109.1 | 76.38 |
| Serratia marcescens BIDMC 44 | Serratia marcescens strain 1274 genome | ETX44761.1 | 77.35 |
| Serratia sp. YD25 | Serratia sp. YD25, complete genome | AOF02338.1 | 93.37 |
| Serratia sp. SCBI | Serratia sp. SCBI, complete genome | AIN23801.1 | 93.44 |
| Serratia marcescens subsp. marcescens AH0650_Sm1 AG2 | Serratia marcescens strain BWH-23 chromosome, complete genome | KMU50701.1 | 99.40 |
| Serratia marcescens subsp. marcescens Db11 | Serratia marcescens subsp. marcescens Db11, complete genome | CDG14244.1 | 99.32 |

Sixty-eight genes were identified in the serrawettin W2 biosynthetic gene clusters (Figure 5 and Table S5). Twenty-four genes were present in all the strains’ gene clusters (Figure 5), namely, the...
genes encoding for RNase E specificity factor CsrD, with aminotransferase activity; acrylyl-CoA reductase; sulfoxide reductase subunit YedY and YedZ; 3-dehydroquinate dehydratase, involved on aromatic amino acids biosynthesis; biotin carboxyl carrier protein and carboxylase subunit, both components of the acetyl-CoA carboxylase complex; sodium/pantothenate symporter; DNA-binding proteins; exported proteins involved in cell adhesion; lipid A biosynthesis lauryl acyltransferase, with catalytic activity; glutamine amidotransferase; murine effectors LrgA and LrgB, with hydrolase activity; and LysR transcriptional regulator, with carbon dehydratase.

The phylogenetic relationship between the strains was established based on the analysis of the concatenated genes of the W2 biosynthetic gene cluster and compared with the relationships defined based on the swrA gene analysis. The same clusters were defined with the swrA phylogenetic tree, showing more homogenous clusters (Figure 5).

The genes coding for alanine racemase, carboxymuconolactone decarboxylase, MFS transporter, hypothetical protein, tautomerase, hypothetical protein, hypothetical protein, LysR family transcriptional regulator, and ssDNA-binding protein (Figure 5, genes numbered from 60 to 68; Table S5) are exclusive to the Serratia sp. YD25T cluster. AntiSMASH cluster prediction identified four additional genes involved in PKS-NRPS, encoding for enoylreductase quinone oxidoreductase and ketoreductase 3-oxoacyl-(acyl-carrier-protein) reductase, both present in all the strains; enoylreductase dehydrogenase, present in seven strains; and aromatic amino acid aminotransferase, present in all strains except in Serratia sp. YD25T (Table 5 and Figure 5).

Table 5. The PKS genes from the swrA biosynthetic gene clusters predicted by antiSMASH software.

| Bacterial Strain               | Enoylreductase Quinone Oxidoreductase | Ketoreductase 3-Oxoyl-(Acyl-Carrier-Protein) Reductase | Enoylreductase Dehydrogenase | Aminotransferase |
|-------------------------------|--------------------------------------|--------------------------------------------------------|------------------------------|------------------|
| Serratia marcescens SM39      | BAO35810.1                           | BAO35835.1                                             | BAO35838.1                   | BAO35842.1       |
| Serratia marcescens SmUNAM836 | ALE98097.1                           | ALE98121.1                                             | ALE98124.1                   | ALE98128.1       |
| Serratia sp. SSNIH1           | AUY16685.1                           | AUY16883.1                                             | AUY16886.1                   | AUY16890.1       |
| Serratia sp. PWNI146          | SAY45233.1                           | SAY45257.1                                             | SAY45263.1                   | SAY45267.1       |
| Serratia ureilytica Lr5/4 LG59| KKO55998.1                           | KKO58361.1                                             | KKO57271.1                   | KKO57271.1       |
| Serratia marcescens RSC-14    | ALD45523.1                           | ALD45509.1                                             | ALD45501.1                   | ALD45501.1       |
| Serratia marcescens BidMC 44  | Etx44746.1                           | Etx44771.1                                             | Etx44774.1                   | Etx44778.1       |
| Serratia sp. YD25             | AOF01119.1                           | AOF01143.1                                             | AOF01143.1                   | Etx44778.1       |
| Serratia sp. SCBI             | AIM23787.1                           | AIM23811.1                                             | AIM23811.1                   | AIM23821.1       |
| Serratia marcescens subsp.    | KMUS0686.1                           | KMUS0711.1                                             | KMUS0716.1                   | KMUS0720.1       |
| Serratia marcescens subsp.    | CDG14228.1                           | CDG14254.1                                             | CDG14259.1                   | CDG14263.1       |
| Serratia marcescens subsp.    | KMUS0686.1                           | KMUS0711.1                                             | KMUS0716.1                   | KMUS0720.1       |
| Serratia marcescens subsp.    | CDG14228.1                           | CDG14254.1                                             | CDG14259.1                   | CDG14263.1       |
| Serratia marcescens subsp.    | KMUS0686.1                           | KMUS0711.1                                             | KMUS0716.1                   | KMUS0720.1       |
| Serratia marcescens subsp.    | CDG14228.1                           | CDG14254.1                                             | CDG14259.1                   | CDG14263.1       |
Figure 5. Serrawettin W2 gene cluster analysis. The phylogenetic relationship was established with Mega X software by the Neighbor-Joining method on aligned serrawettin W1 gene clusters of *Serratia* strains PWN146, SSNIH1, SM39, SmUNAm836, BIDMC44, Lr5/4 LG59, RSC-14, AH0650, Db11, SCBI, and YD25T. (a) Phylogenetic tree based on protein sequences of serrawettin W2 biosynthetic gene clusters on an established genetic organization. (b) Serrawettin W2 biosynthetic gene clusters based on natural genetic organization. (c) Comparison of the phylogenetic tree based on protein sequences of the serrawettin W1 biosynthetic gene clusters (left) with the phylogenetic tree of the serrawettin W1 biosynthesis protein (right). The scale bar of 0.050 infers nucleotide substitutions per 100 nucleotides.
4. Discussion

Strains of the genus *Serratia* are known to colonize a diversity of environments. This ability is usually related to the ability to produce lipopeptides, which includes the serrawettins. The physiological roles of such surface-active exolipids are mostly unknown but seem to contribute specifically to enhancing the flagellum-dependent and flagellum-independent spreading growth of the bacteria on a surface environment. Serrawettins were first reported in pigmented *S. marcescens* [4] and 53.6% of *Serratia* strains that showed the *swrW* or *swrA* genes clusters in our study belonged to this species. The analysis of the genomes of 84 *Serratia* strains confirmed that *S. marcescens* is the only species with strains able to produce serrawettin W1 or W2. From the total genomes analyzed, the *swrW* biosynthetic gene clusters were detected in 17 *Serratia* genomes. These strains belonged to different species, namely, eight *S. marcescens*, four *S. plymuthica*, two *S. grimesii*, one *S. nematodiphila*, and two unidentified *Serratia* species. Eleven *Serratia* genomes showed *swrA* biosynthetic gene clusters. The *swrA* biosynthetic gene clusters were detected in strains belonging to the species *S. marcescens* (seven), *S. ureilytica* (one), and three belong to unidentified *Serratia* species. These strains come from different sources, such as waste water [43], paper machines [34], infected patients [19,44,49,60], pond water, human tissue [35], rapeseed roots [29–31], nematodes, buffer solutions [25], different plants [39,61], and soil [27,28].

AntiSMASH software was able to identify the serrawettins gene clusters as NRPS clusters, as previously described [12,13]. When compared with the known serrawettin W1 proteins, the *S. marcescens* strains had a protein with an identity percentage higher than 95%, while proteins of the *S. plymuthica* and *S. grimesii* strains showed an identity higher than 81% and 78%, respectively. From the serrawettin W1 biosynthesis proteins, 70.2% are conserved residues present in all core biosynthetic proteins, suggesting that the serrawettin W1 protein is well conserved among different strains. When compared with the known serrawettin W2 proteins, *S. marcescens* and *S. ureilytica* showed a protein identity higher than 76%, *Serratia* strains YD25 and SCBI higher than 93%, and subspecies of *S. marcescens* higher than 99%. In spite of *swr2* proteins divergence, 62.3% were conserved residues present in all core serrawettin W2 biosynthetic proteins, suggesting a well-conserved serrawettin W2 protein among the different strains.

As previously described by Li et al. [12], all serrawettin W1 biosynthetic genes have a condensation, adenylation, thiolation, and thioesterase domains, also predicted in our study by antiSMASH software. From the serrawettin W1 biosynthetic gene clusters, a total of 80 different genes were identified, 15 common to all clusters. These common genes encode for proteins that may play an important role in serrawettin W1 biosynthesis. AntiSMASH software predicted four PKS-NRPS genes in the serrawettin W1 cluster, which are multi-enzymatic and multi-domain genes involved in the biosynthesis of nonribosomal peptides: an oxoreductase present in 7 *Serratia* clusters (enoylreductase), a 3-oxoacyl-(acyl-carrier-protein) reductase present in all *Serratia* clusters (ketoreductase), a dehydrogenase present in 14 *Serratia* clusters (enoylreductase), and an aromatic amino acid aminotransferase present in 15 *Serratia* clusters (aminotransferase). Usually, PKS genes consist of the aminotransferase (AT), ketosynthase (KS), and a ketoreductase (KR) domains [13]. Proteins of the serrawettin W1 biosynthetic gene cluster tree and *SwrW* biosynthesis protein tree, when compared, revealed common clades, suggesting that the *swrW* gene and all cluster organizations have a similar evolutionary history.

Analysis of the *swrA* biosynthetic gene clusters from 11 *Serratia* genomes demonstrated complex serrawettin W2 biosynthesis protein domains with five condensation domains, five adenylation domains, five thiolation domains, and one thioesterase domain, as previously described in Su et al. [13]. From the *swrA* biosynthetic gene clusters, a total of 68 different proteins were identified, 24 common to all clusters, and 8 exclusive to the *Serratia* sp. YD25 biosynthetic gene cluster. These common proteins may play an important role in serrawettin W2 biosynthesis and may be involved in the different steps needed to produce a nonribosomal peptide as DNA binding, adenylation, condensation, thiolation, and thioesterase. According to Su et al. [13], the three proteins identified as involved in PKS-NRPS hybrid polyketide synthase (acyltransferase, ketosynthase, and ketoreductase) are also
involved in the serrawettin W2 biosynthesis process. Our results of the serrawettin W2 biosynthetic gene clusters revealed four genes identified as part of the PKS-NRPS system, such as oxireductase and 3-oxoacyl-(acyl-carrier-protein) reductase, both present in all serrawettin W2 biosynthesis protein clusters; a dehydrogenase present in seven clusters; and an aromatic amino acid aminotransferase present in nine clusters. Proteins of the serrawettin W2 gene clusters tree and SwrA biosynthesis protein tree, when compared, revealed common groups in both trees, suggesting that not only the serrawettin W2 biosynthesis gene but also all cluster organizations have a similar evolutionary history.

None of the genomes analyzed included both the serrawettin biosynthetic gene clusters. Although both present in the \textit{Serratia} genus, the two biosynthetic gene clusters are distributed in the two clades, as revealed in the 16S rRNA gene-defined phylogenetic tree of the genus \textit{Serratia}. W1 is present in two clades, in five \textit{S. marcescens}, one \textit{S. nematodiphila}, and in all the strains of the genus \textit{S. plymuthica} analyzed, except one, and in two \textit{S. grimesii} and one \textit{S. rubidaea}. On the other hand, W2 is common in all strains of the \textit{S. marcescens} sub-cluster and three additional strains of the same species, but it was not present in the other \textit{Serratia} clade.

This work identified four genes common to all serrawettin gene clusters, highlighting their essential potential in the serrawettins biosynthetic process. These genes encoding for CTP synthase, glyoxalase/bleomycin resistance protein/dioxygenase, LrgA family protein, and LrgB family protein are flanking the biosynthesis genes \textit{swrW} and \textit{swrA}. In both organizations, the genes encoding for CTP synthase is immediately upstream of the serrawettin biosynthetic gene and the ones encoding for the LrgA and LrgB family proteins are immediately downstream of the genes. Glyoxalase/bleomycin resistance protein/dioxygenase is downstream of \textit{swrW} and \textit{swrA}, with a group of non-conserved genes between them. The \textit{lrgAB} operon in the \textit{Staphylococcus} codes for a transmembrane protein. The LrgA protein shares many characteristics with bacteriophage antiholins [62]. The antiholin homologue in \textit{Bacillus subtilis} transports pyruvate and it is regulated in an unconventional way by its substrate molecule [63]. Holins and antiholins control the formation of channels for murein hydrolase to export across the bacterial membrane to time the bacteriophage-induced cell lysis [64]. In \textit{Serratia}, this operon seems to be associated with the transport of serrawettin, as a facilitator, independently of the coding gene (\textit{swrW} or \textit{swrA}), and, therefore, of the complexity of the molecule. The holing-antiholin class of proteins was originally discovered in bacteriophages, where they modulate host cell lysis during lytic infection [65]. A hypothetical model suggests that these proteins could have been acquired by horizontal gene transfer to an ancient bacterium through the integration of these elements into its genome [66]. This suggests the introduction of \textit{swrW} and \textit{swrA} as two independent events in the \textit{lrgAB} operon, but more work is needed to understand the evolution and functional diversification of serrawettin.

Within the W1 biosynthetic gene cluster, the gene coding for a quinone oxidoreductase YhdH/YhfP family is present only in two strains of \textit{S. marcescens} and in three strains from different species of \textit{Serratia}. These \textit{Serratia} strains are missing the gene cluster characterized by the Major Facilitator Superfamily (MFS) gene present in all the other \textit{S. marcescens} strains. MFS is one of the two largest families of membrane transporters found in bacteria [67]. Phylogenetic analyses revealed the occurrence of 17 distinct families within the MFS, each of which generally transports a single class of compounds. This suggests that within the W1 cluster, the MFS transport system is conserved for most \textit{S. marcescens} although the other \textit{Serratia} species presented the quinone oxidoreductase system YhdH.

The W2 biosynthetic gene cluster has a more conserved genetic organization and 24 genes were common to all strains. The gene clusters composed of cystine ABC transporter, substrate-binding protein, and the alanine racemase seem to be involved in the selective transport of amino acids into the cell and in the alanine L to D interconversion. These systems may facilitate the amino acid acquisition by the cell for W2 synthesis. In the W2 biosynthetic gene cluster, both genes are present in several \textit{Serratia} species but only in four strains of \textit{S. marcescens}. Both the mechanistic studies, kinetic and energetic, are needed to relate the genes’ presence with W2 synthesis in these strains. The other four strains producing the serrawettin W2, which do not present the gene cluster with ABC transporter and
racemase, show a gene cluster including a gene belonging to the cyclase family protein. In Serratia, the cyclase family protein shows high homology with diguanylate cyclase, showing a domain from the GGDEF family protein [68]. They are used as an intracellular signaling molecule regulator, involved in bacterial biofilm formation, and persist in several bacteria species.

5. Conclusions

In conclusion, the present work shows that most species of the genus Serratia that already have their genome sequenced have clusters of serrawettin biosynthetic genes in their genomes. AntiSMASH software was able to identify the serrawettins gene clusters as NRPS clusters. The grouping of biosynthetic gene clusters W1 and W2 are mutually exclusive in the genome. Moreover, the surW and surA biosynthetic genes are framed by the same genes in the biosynthetic clusters. CTP is upstream and the operon LgrAB is downstream, suggesting a horizontal gene acquisition of the biosynthetic system by an ancient strain. Within the W1 biosynthetic cluster, the genes coding for the quinone oxidoreductase YhdH/YhfP family, and the one coding for the Major Facilitator Superfamily, are mutually exclusive in the genomes of the strains. The same is found in the W2 biosynthetic gene cluster, in the genes cystine ABC transporter, substrate-binding protein, and the alanine racemase and cyclase family proteins are also mutually exclusive.

Supplementary Materials: The following are available online at http://www.mdpi.com/2079-7737/9/12/482/s1, Table S1. General information of Serratia strains used in this study. Table S2. Serratia W1 gene (surW) of each strain predicted by antiSMASH in Serratia strains ATCC 13880, CDC_813-60 DP21, UMH8, IOMTU 115, DSM 21420, VGH107, EG2-HP20, WW4, FS14, BIDMC81, TEL NODE_13, NBRC 102599, BXF1, A2, AS13, AS9 and AS12, in this study, and the accession numbers and identification of surW closest relatives using BLASTP. Table S3. Serratia W2 gene (surA) of each strain predicted by antiSMASH in Serratia strains PWN146, SSNIH1, SM39, SmUNAm836, BIDMC44, Lr5/4 LS59, RSC-14, AH0650, Db11, SCBI and YD25, in this study, and the accession numbers and identification of surA closest relatives using BLASTX. Table S4. Accession numbers of identified proteins of serrawettin W1 biosynthetic gene clusters of Serratia strains ATCC 13880, CDC_813-60 DP21, UMH8, IOMTU 115, DSM 21420, VGH107, EG2-HP20, WW4, FS14, BIDMC81, TEL NODE_13, NBRC 102599, BXF1, A2, AS13, AS9 and AS12 by antiSMASH and NCBI BLASTP software. Table S5. Accession numbers of identified proteins of serrawettin W2 biosynthetic gene clusters of Serratia strains PWN146, SSNIH1, SM39, SmUNAm836, BIDMC44, Lr5/4 LS59, RSC-14, AH0650, Db11, SCBI and YD25 by antiSMASH and NCBI BLASTX software.

Author Contributions: Conceptualization, D.N.P. and P.V.M.; Methodology, C.M.-P. and D.N.P.; Software, C.M.-P. and D.N.P.; Formal analysis, C.M.-P. and D.N.P.; Investigation, C.M.-P. and D.N.P.; Resources, P.V.M.; data curation, D.N.P. and P.V.M.; Writing—Original draft preparation, C.M.-P., D.N.P. and P.V.M.; Writing—review and editing, D.N.P. and P.V.M.; Supervision, D.N.P. and P.V.M.; Project administration, P.V.M.; Funding acquisition, P.V.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by FEDER funds through the program COMPETE - Programa Operacional Factores de Competitividade - and by national funds through Fundação para a Ciência e a Tecnologia (FCT), under the projects UID/EMS/00285/2020, PTWPTDC/AAG-REC/3839/2014; and by BIORECOVER Horizon2020 under grant agreement No 821096.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Araujo, H.W.C.; Andrade, R.F.S.; Montero-Rodriguez, D.; Rubio-Ribeaux, D.; Alves Da Silva, C.A.; Campos-Takaki, G.M. Sustainable biosurfactant produced by Serratia marcescens UCP 1549 and its suitability for agricultural and marine bioremediation applications. Microb. Cell Fact. 2019, 18, 2. [CrossRef]
2. Mujumdar, S.; Bashetti, S.; Pardeshi, S.; Thombre, R.S. Industrial applications of biosurfactants. In Industrial Biotechnology; Apple Academic Press: Palm Bay, FL, USA, 2017; pp. 81–109.
3. Nitschke, M.; Silva, S.S. Recent food applications of microbial surfactants. Crit. Rev. Food Sci. Nutr. 2018, 58, 631–638. [CrossRef]
4. Matsuyama, T.; Kaneda, K.; Nakagawa, Y.; Isa, K.; Hara-Hotta, H.; Yano, I. A novel extra cellular cyclic lipopeptide which promotes flagellum-dependent and -independent spreading growth of Serratia marcescens. J. Bacteriol. 1992, 174, 1769–1776. [CrossRef] [PubMed]
5. Hajfarajollah, H.; Eslami, P.; Mokhtarani, B.; Akbari Naghabi, K. Biosurfactants from probiotic bacteria: A review. Biotechnol. Appl. Biochem. 2018, 65, 768–783. [CrossRef] [PubMed]
6. Sharafi, H.; Abdoli, M.; Hajfarajollah, H.; Samie, N.; Alidoust, L.; Abbasi, H.; Fooladi, J.; Zahiri, H.S.; Noghabi, K.A. First report of a lipopeptide biosurfactant from thermophilic bacterium Anearinnicellis thermoacrophilus MK01 newly isolated from municipal landfill site. Appl. Biochem. Biotechnol. 2014, 173, 1236–1249. [CrossRef] [PubMed]

7. Grimont, P.A.D.; Grimont, F. The genus Serratia. Annu. Rev. Microbiol. 1978, 32, 221–248. [CrossRef] [PubMed]

8. Fallis, A. Proteobacteria Gamma; Springer: New York, NY, USA, 2013; Volume 53, ISBN 9788578110796.

9. Mahlen, S.D. Serratia infections: From military experiments to current practice. Clin. Microbiol. Rev. 2011, 24, 755–791. [CrossRef] [PubMed]

10. Wasserman, H.H.; Keggi, J.J.; McKeon, J.E. The structure of serratamolide. [CrossRef] [PubMed]

11. Matsuyama, T.; Murakami, T.; Fujita, M.; Fujita, S.; Yano, I. Extracellular vesicle formation and biosurfactant production by Serratia marcescens. Microbiology 1986, 132, 865–875. [CrossRef]

12. Li, H.; Tanikawa, T.; Sato, Y.; Nakagawa, Y.; Matsuyama, T. Serratia marcescens gene required for surfactant serrawettin W1 production encodes putative aminolipid synthetase belonging to nonribosomal peptide synthetase family. Microb. Immunol. 2005, 49, 303–310. [CrossRef]

13. Su, C.; Xiang, Z.; Liu, Y.; Zhao, X.; Sun, Y.; Li, Z.; Li, L.; Chang, F.; Chen, T.; Wen, X.; et al. Analysis of the genomic sequences and metabolites of Serratia surfactantifaciens sp. nov. YD25T that simultaneously produces prodigiosin and serrawettin W2. BMC Genom. 2016, 17, 1–19. [CrossRef] [PubMed]

14. Pradel, E.; Zhang, Y.; Pujol, N.; Matsuyama, T.; Bargmann, C.I.; Ewbank, J.J. Detection and avoidance of a natural product from the pathogenic bacterium Serratia marcescens by Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 2007, 104, 2295–2300. [CrossRef] [PubMed]

15. Clements, T.; Ndlovu, T.; Khan, W. Broad-spectrum antimicrobial activity of secondary metabolites produced by Serratia marcescens strains. Microbiol. Res. 2019, 229, 126329. [CrossRef] [PubMed]

16. Eckelmann, D.; Spiteller, M.; Kusari, S. Spatial-temporal profiling of prodiginines and serratamolides produced by endophytic Serratia marcescens harbored in Maytenus serrata. Sci. Rep. 2018, 8, 1–15. [CrossRef]

17. Anzuay, M.S.; Frola, O.; Angelini, J.G.; Ludueña, L.M.; Fabra, A.; Taurian, T. Genetic diversity of phosphate-solubilizing peanut (Arachis hypogaea L.) associated bacteria and mechanisms involved in this ability. Symbiosis 2013, 60, 143–154. [CrossRef]

18. Park, S.K.; Kim, Y.C. Draft genome sequence of a chitinase-producing biocontrol bacterium Serratia sp. C-1. Res. Plant Dis. 2015, 21, 222–226. [CrossRef]

19. Sandner-Miranda, L.; Vinuesa, P.; Soberón-Chávez, G.; Morales-Espinosa, R. Complete genome sequence of Serratia marcescens SmUNAM836, a nonpigmented multidrug-resistant strain isolated from a Mexican patient with obstructive pulmonary disease. Genome Announc. 2016, 4, 1–2. [CrossRef]

20. Aylward, F.O.; Tremmel, D.M.; Starrett, G.J.; Bruce, D.C.; Chain, P.; Chen, A.; Davenport, K.W.; Detter, C.; Han, C.S.; Han, J.; et al. Complete genome of Serratia sp. strain FG1 94, a strain associated with leaf-cutter ant fungus gardens. Genome Announc. 2013, 1, e00239-12. [CrossRef]

21. Proenza, D.N.; Santo, C.E.; Grass, G.; Morais, P.V. Draft genome sequence of Serratia sp. strain M24T3, isolated from pinewood disease nematode Bursaphelenchus xylophilus. J. Bacteriol. 2012, 194, 3764. [CrossRef]

22. Vicente, C.S.L.; Nascimento, F.X.; Barbosa, P.; Ke, H.-M.; Tsai, I.J.; Hirao, T.; Cock, P.J.A.; Kikuchi, T.; Hasegawa, K.; Mota, M. Evidence for an opportunistic and endophytic lifestyle of the Bursaphelenchus xylophilus-associated bacteria Serratia marcescens PWN146 isolated from wilting Pinus pinaster. Appl. Biochem. Biotechnol. 2013, 173, 1–19. [CrossRef] [PubMed]

23. Vicente, C.S.L.; Nascimento, F.X.; Ikuyo, Y.; Cock, P.J.A.; Mota, M.; Hasegawa, K. The genome and genetics of a high oxidative stress tolerant Serratia sp. LCN16 isolated from the plant parasitic nematode Bursaphelenchus xylophilus. BMC Genom. 2016, 17, 1–15. [CrossRef] [PubMed]

24. Nascimento, F.; Vicente, C.; Cock, P.; Tavares, M.; Rossi, M.; Hasegawa, K.; Mota, M. From plants to nematodes: Serratia grimesii BXF1 genome reveals an adaptation to the modulation of multi-species interactions. Microb. Genom. 2018, 4. [CrossRef] [PubMed]

25. Mardanova, A.M.; Toymenstateva, A.A.; Gilyazeva, A.G.; Kazakov, S.V.; Shagimardanova, E.I.; Khaitlina, S.Y.; Sharipova, M.R. Draft genome sequence of Serratia grimesii strain A2. Genome Announc. 2014, 2, 7–8. [CrossRef] [PubMed]
45. Pei, D.; Hill-Clemens, C.; Carissimo, G.; Yu, W.; Vernick, K.D.; Xu, J. Draft genome sequences of two strains of *Serratia* spp. from the midgut of the malaria mosquito *Anopheles gambiae*. *Genome Announc.* 2016, 3, e00990-15. [CrossRef] [PubMed]

46. Poehlein, A.; Freese, H.M.; Daniel, R.; Simeonova, D.D. Draft genome sequence of *Serratia* sp. strain DD3, isolated from the guts of *Daphnia magna*. *Genome Announc.* 2014, 2, e00903-14. [CrossRef] [PubMed]

47. Chen, X.; Hitchings, M.D.; Mendoza, J.E.; Balanza, V.; Facey, P.D.; Dyson, P.J.; Bielza, P.; Del Sol, R. Comparative genomics of facultative bacterial symbionts isolated from *European orius* species reveals an ancestral symbiotic association. *Front. Microbiol.* 2017, 8, 1969. [CrossRef] [PubMed]

48. Nicholson, W.L.; Leonard, M.T.; Fajardo-Cavazos, P.; Panayotova, N.; Farmerie, W.G.; Tripplett, E.W.; Schuerger, A.C. Complete genome sequence of *Serratia liquefaciens* strain ATCC 27592. *Genome Announc.* 2013, 1, e00548-13. [CrossRef] [PubMed]

49. Iguchi, A.; Nagaya, Y.; Pradel, E.; Ooka, T.; Ogura, Y.; Katsura, K.; Kurokawa, K.; Oshima, K.; Hattori, M.; Weber, T.; Blin, K.; Duddela, S.; Krug, D.; Kim, H.U.; Bruccoleri, R.; Lee, S.Y.; Fischbach, M.A.; Müller, R.; Wohlleben, W.; et al. Introducing EzTaxon-e: A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* 2012, 62, 716–721. [CrossRef]

50. Kelley, L.A.; Mezulis, S.; Yates, C.M.; Wass, M.N.; Sternberg, M.J.E. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* 2015, 10, 845–888. [CrossRef]

51. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 2018, 35, 1547–1549. [CrossRef] [PubMed]

52. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 1987, 4, 406–425. [PubMed]

53. Altschul, S.F.; Madden, T.L.; Schä, W.; et al. PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 1997, 25, 3389–3402. [CrossRef]

54. Ludwig, W.; Strunk, O.; Westram, R.; Richter, L.; Meier, H.; Yadhukumar; Buchner, A.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 1997, 25, 3389–3402. [CrossRef]

55. Stamatakis, A. RAXML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 2006, 22, 2688–2690. [CrossRef] [PubMed]

56. Kelley, L.A.; Mezulis, S.; Yates, C.M.; Wass, M.N.; Sternberg, M.J.E. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* 2015, 10, 845–888. [CrossRef]

57. Pruesse, E.; Pevleis, J.; Glöckner, F.O. SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012, 28, 1823–1829. [CrossRef]

58. Ludwig, W.; Strunk, O.; Westram, R.; Richter, L.; Meier, H.; Yadhukumar; Buchner, A.; Lipman, D.J. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 1997, 25, 3389–3402. [CrossRef]

59. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 1987, 4, 406–425. [PubMed]

60. UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Res.* 2019, 47, D506–D515. [CrossRef] [PubMed]

61. Nascimento, F.X.; Espada, M.; Barbosa, P.; Rossi, M.J.; Vicente, C.S.L.; Mota, M. Non-specific transient envelope membrane protein containing a putative LrgB domain related to the control of bacterial death and lysis is required for chloroplast development in *Bursaphelenchus xylophilus*. *Nucleic Acids Res.* 2019, 47, D506–D515. [CrossRef] [PubMed]

62. Yang, Y.; Jin, H.; Chen, Y.; Lin, W.; Wang, C.; Chen, Z.; Han, N.; Bian, H.; Zou, M.; Wang, J. A chloroplast envelope membrane protein containing a putative LrgB domain related to the control of bacterial death and lysis is required for chloroplast development in *Arabidopsis thaliana*. *New Phytol.* 2012, 193, 81–95. [CrossRef]

63. Charbonnier, T.; Le Coq, D.; McGovern, S.; Calabre, M.; Delumeau, O.; Aymerich, S.; Jules, M. Molecular and physiological logics of the pyruvate-induced response of a novel transporter in *Bacillus subtilis*. *MBio* 2017, 8, e00976-17. [CrossRef] [PubMed]

64. Wang, I.-N.; Smith, D.L.; Young, R. Holins: The protein clocks of bacteriophage infections. *Annu. Rev. Microbiol.* 2000, 54, 799–825. [CrossRef]
65. Ahn, S.-J.; Rice, K.C.; Oleas, J.; Bayles, K.W.; Burne, R.A. The Streptococcus mutans Cid and Lrg systems modulate virulence traits in response to multiple environmental signals. *Microbiology* 2010, 156, 3136–3147. [CrossRef] [PubMed]

66. van den Esker, M.H.; Kovács, Á.T.; Kuipers, O.P. From cell death to metabolism: Holin-antiholin homologues with new functions. *MBio* 2017, 8, e01963-17. [CrossRef] [PubMed]

67. Pao, S.S.; Paulsen, I.T.; Saier, M.H. Major facilitator superfamily. *Microbiol. Mol. Biol. Rev.* 1998, 62, 1–34. [CrossRef]

68. D’Argenio, D.A.; Miller, S.I. Cyclic di-GMP as a bacterial second messenger. *Microbiology* 2004, 150, 2497–2502. [CrossRef] [PubMed]

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).