

Complete genome sequence of *Serratia plymuthica* strain AS12

Saraswoti Neupane¹, Roger D. Finlay¹, Sadhna Alström¹, Lynne Goodwin^{2,3}, Nikos C. Kyrpides², Susan Lucas², Alla Lapidus², David Bruce^{2,3}, Sam Pitluck², Lin Peters², Galina Ovchinnikova², Olga Chertkov^{2,3}, James Han², Cliff Han^{2,3}, Roxanne Tapia^{2,3}, John C. Detter^{2,3}, Miriam Land^{2,4}, Loren Hauser^{2,4}, Jan-Fang Cheng², Natalia Ivanova², Ioanna Pagani², Hans-Peter Klenk⁵, Tanja Woyke² and Nils Högberg¹

¹ Uppsala BioCenter, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden

² DOE Joint Genome Institute, Walnut Creek, California, USA,

³ Los Alamos National Laboratory, Bioscience Division, Los Alamos, New Mexico, USA,

⁴ Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

⁵ Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Corresponding author: Saraswoti Neupane

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A plant-associated member of the family *Enterobacteriaceae*, *Serratia plymuthica* strain AS12 was isolated from rapeseed roots. It is of scientific interest because it promotes plant growth and inhibits plant pathogens. The genome of *S. plymuthica* AS12 comprises a 5,443,009 bp long circular chromosome, which consists of 4,952 protein-coding genes, 87 tRNA genes and 7 rRNA operons. This genome was sequenced within the 2010 DOE-JGI Community Sequencing Program (CSP2010) as part of the project entitled “Genomics of four rapeseed plant growth promoting bacteria with antagonistic effect on plant pathogens”.

Introduction

Plant associated *Serratia* species are commonly found as free-living bacteria in rhizosphere soil and as endophytes within plant roots. They include strains with the ability to stimulate plant growth and to inhibit the growth of soil borne pathogens of economically important agricultural plants [1-3]. One *Serratia* strain, *S. plymuthica* HRO-C48, is successfully used as an alternative to chemical agents for control of soil-borne fungal diseases in different crops such as strawberry and rapeseed [3,4]. Its ability to degrade chitin, a fungal cell wall component, may be responsible for antifungal activity, whereas the production of the plant hormone indole-3-acetic acid (IAA) could be involved in plant growth promotion [3]. *S. plymuthica* AS12 has chitinolytic activity and was isolated from rapeseed roots from Uppsala, Sweden in 1998 [5]. The reason for our interest in *S. plymuthica* AS12 is its ability to inhibit *Verticillium longisporum* (earlier *V. dahliae*), a soil borne fungal pathogen of rapeseed, thus promoting the rapeseed growth both directly and indirectly [5].

Here we present a description of the complete genome of *S. plymuthica* AS12 and its annotation.

Classification and features

A representative 16S rRNA gene sequence of the strain AS12 genome was used for comparison using NCBI BLAST [6] under default settings with the most recent databases. The relative frequencies of taxa and BLAST scores were determined. The most frequently occurring genus is *Serratia* where some of the ‘hits’ share a 100% identity. When considering high-scoring segment pairs (HSPs) from the best 250 hits, the most frequent matches were *Serratia sp.* (17.2%) with a maximum identity of 97-100%, while *S. plymuthica* (5.2%) had a maximum identity of 97-100%, *S. proteomaculans* (4.8%) with a maximum identity of 97-99%, *S. marcescens* (4.8%) with a maximum identity of 96-97% and different strains of *Rahnella* (7%) with a maximum identity of 97-98%.

A phylogenetic tree (Figure 1) was constructed using 16S rRNA sequences of *S. plymuthica* AS12 with other genera within the family *Enterobacteriaceae* including two species within the genus *Serratia*. The tree shows the position of *S. plymuthica* AS12 within the genus *Serratia* and its distinct clustering with *S. plymuthica*, which was confirmed by digital DNA-DNA hybridization values [11] above 70% with the (unpublished) draft genome sequence of the *S. plymuthica* type strain Breed K-7^T from a DSM 4540 culture as well as with the complete genome sequence of *S. plymuthica* AS9 [12] using the GGDC web server [13].

The cells of strain AS12 stain Gram-negative and are rod shaped, 1-2 µm long, 0.5-0.7 µm wide (Figure 2 and Table 1) and motile. The culture forms red to pink colored colonies of 1-2 mm diameter on tryptic soy agar and potato dextrose agar, but the colony color depends on different factors such as the growth substrate, pH of the medium and growth temperature. The organism is a facultative anaerobe and grows at temperatures ranging from

4 °C - 40 °C and within a pH range of 4 - 10. It has the ability to utilize a wide range of carbon sources such as glucose, sucrose, succinate, mannitol and arabinose. It also has cellulolytic, phospholytic, chitinolytic and proteolytic activity [5]. The strain is deposited in the Culture Collection, University of Göteborg, Sweden (CCUG) as *Serratia sp.* AS12 (= CCUG 61397).

Chemotaxonomy

The cells of *S. plymuthica* AS12 contain a mixture of saturated and unsaturated fatty acids. The dominant fatty acids in strain AS12 are C_{16:0} (22.94%), C_{16:1ω7c} (17.08%), C_{18:1ω7c} (19.65%), C_{14:0} (5.11%), along with other minor fatty acid components. No information is available for other compounds. Previously it has been shown that *Serratia* spp. contain a mixture of C_{14:0}, C_{16:0}, C_{16:1} and C_{18:1+2} fatty acids in which 50-80% of the total fatty acid in the cell is C_{14:0} and others each less than 3% [28]. This is consistent with the observation that C_{14:0} is a characteristic fatty acid of the family *Enterobacteriaceae*.

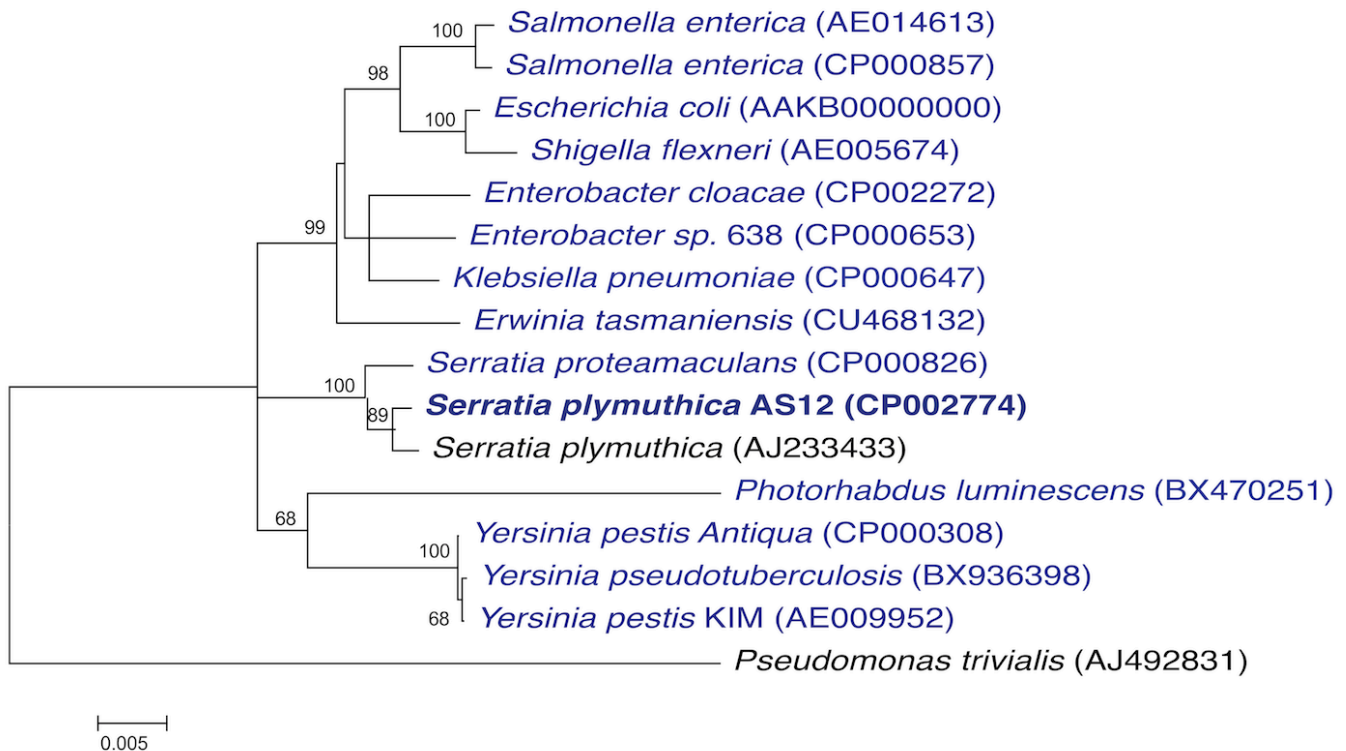


Figure 1. Phylogenetic tree highlighting the position of *S. plymuthica* AS12 in relation to selected *Serratia* strains and other genera within the family *Enterobacteriaceae*. The tree was based on 1,535 characters of the 16S rRNA gene sequence aligned in ClustalW2 [7]. The tree was inferred under the maximum likelihood criterion using MEGA5 software [8] and rooted with *Pseudomonas trivialis* (a member of the *Pseudomonadaceae* family). The branches are mapped by the expected number of substitutions per site. The numbers above the branches are support values from 1,000 bootstrap replicates if larger than 60% [9]. Lineages with genome sequences registered in GOLD [10] are shown in blue.

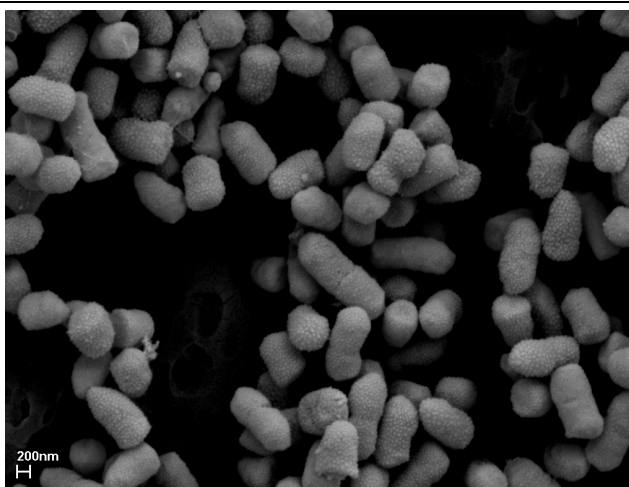


Figure 2. Scanning electron micrograph of *S. plymuthica* AS12

Table 1. Classification and general features of *S. plymuthica* AS12 according to MIGS recommendations [14]

MIGS ID	Property	Term	Evidence code ^a
	Current classification	Domain <i>Bacteria</i>	TAS [15]
		Phylum <i>Proteobacteria</i>	TAS [16]
		Class <i>Gammaproteobacteria</i>	TAS [17,18]
		Order " <i>Enterobacteriales</i> "	TAS [19]
		Family <i>Enterobacteriaceae</i>	TAS [20-22]
		Genus <i>Serratia</i>	TAS [20,23,24]
		Species <i>Serratia plymuthica</i>	TAS [20,25]
		Strain AS12	IDA
	Gram stain	Negative	IDA
	Cell shape	Rod-shaped	IDA
	Motility	Motile	IDA
	Sporulation	Non-sporulating	IDA
	Temperature range	Mesophilic, 4 – 40°C	IDA
	Optimum temperature	28°C	IDA
	Carbon source	Glucose, sucrose, fructose, succinate, trehalose, mannitol, inositol, arabinose	IDA
	Energy metabolism	Chemoorganotrophic	IDA
MIGS-6	Habitat	Rapeseed roots	IDA
MIGS-6.3	Salinity	Medium	IDA
MIGS-22	Oxygen	Facultative	IDA
MIGS-15	Biotic relationship	Endophyte	TAS [5]
MIGS-14	Pathogenicity	None	NAS
	Biosafety level	1+	TAS [26]
MIGS-4	Geographic location	Uppsala, Sweden	NAS
MIGS-5	Sample collection time	Summer 1998	NAS
MIGS-4.1	Latitude	59.8	NAS
MIGS-4.2	Longitude	17.65	NAS
MIGS-4.3	Depth	0.1 m	NAS
MIGS-4.4	Altitude	24-25 m	NAS

a) Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [27]. If the evidence code is IDA, then the property should have been directly observed, for the purpose of this specific publication, for a live isolate by one of the authors, or an expert or reputable institution mentioned in the acknowledgements.

Genome sequencing information

S. plymuthica AS12 was selected for sequencing on the basis of its ability to promote rapeseed plant growth as well as to inhibit fungal pathogens of rapeseed [5]. The genome sequence is deposited in the Genomes On Line Database [10] (GOLD ID = Gc01771) and in GenBank (INSDC ID = CP002774). Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information and its association with MIGS identifiers is shown in Table 2.

Growth conditions and DNA isolation

The cells of *S. plymuthica* AS12 were grown in Luria Broth (LB) medium at 28°C with constant shaking at 200 rpm. The cells were harvested after 12 hours when the cells were in the early stationary phase. The cells were pelleted and resuspended in TE buffer (Sigma Aldrich). The DNA was extracted from the resuspended cells by following the standard CTAB protocol for bacterial genomic DNA isolation, which is available at JGI [29].

Table 2. Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	Three libraries: one 454 standard library, one paired end 454 library (12 kb insert size) and one Illumina library
MIGS-29	Sequencing platforms	Illumina GAii, 454 GS FLX Titanium
MIGS-31.2	Fold coverage	59.0 × Illumina; 8.8 × pyrosequencing
MIGS-30	Assemblers	Velvet v. 1.0.13, Newbler v. 2.3, Phrap version SPS – 4.24
MIGS-32	Gene calling method	Prodigal 1.4, GenePRIMP
	NCBI project ID	60453
	INSDC ID	CP002774
	Genbank Date of Release	October 12, 2011
	GOLD ID	Gc01771
MIGS-13	Source material identifier	CCUG 61397
	Project relevance	Biocontrol, Agricultural

Genome sequencing and assembly

The genome of *S. plymuthica* AS12 was sequenced using a combination of Illumina [30] and 454 sequencing platforms [31]. The detailed information on library construction and sequencing can be found at the JGI website [29]. The sequence data from Illumina GAii (1,800 Mb) were assembled with Velvet [32] and the consensus sequence was computationally shredded into 1.5 kb overlapping fake reads. The sequencing data from 454 pyrosequencing (81.6 Mb) were assembled with Newbler. The initial draft assembly contained 61 contigs in one scaffold and consensus sequences were computationally shredded into 2 kb overlapping fake reads. The 454 Newbler consensus reads, the Illumina Velvet consensus reads and the read pairs in the 454 paired end library were integrated using a software parallel Phrap [33]. Possible mis-assemblies were corrected with gapResolution [29], Dupfinisher [34], or by sequencing cloned bridging PCR fragments with

subcloning or transposon bombing (Epicentre Biotechnologies, Madison, WI). The gaps between contigs were closed by editing in the software Consed [35-37], by PCR and by Bubble PCR (J.-F. Chang, unpublished) primer walks. A total of 160 additional reactions was necessary to close gaps and to raise the quality of the finished sequence. The sequence reads from Illumina were used to correct potential base errors and increase consensus quality using the software Polisher developed at JGI [38]. The final assembly is based on 47.4 Mb of 454 draft data which provides an average 8.8 × coverage of the genome and 315 Mb of Illumina draft data which provides an average 59 × coverage of the genome.

Genome annotation

The *S. plymuthica* AS12 genes were identified using Prodigal [39] as part of the genome annotation pipeline at Oak Ridge National Laboratory

(ORNL), Oak Ridge, TN, USA, followed by a round of manual curation using the JGI GenePRIMP pipeline [40]. The predicted CDS were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, Uniport, TIGR-Fam, Pfam, PRIAM, KEGG, COG and InterPro databases. The miscellaneous functions were predicted using tRNAscan-SE [41], RNAmmer [42], TMHMM [43], and signalP [44]. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes – Expert Review (IMG-ER) platform developed by the Joint Genome Institute, Walnut Creek, CA, USA [45].

Genome properties

The genome of *S. plymuthica* AS12 comprises a single circular chromosome of 5,443,009 bp with 55.96% GC content (Figure 3 and Table 3) and 5,140 predicted genes. Among those predicted genes, 4,952 were assigned as protein-coding genes and 88.71% of protein coding genes were assigned for putative function and the remaining ones were annotated as hypothetical proteins. There were 76 pseudogenes and 113 RNA genes with seven rRNA operons. The distribution of genes into the COG functional categories is presented in Table 4.

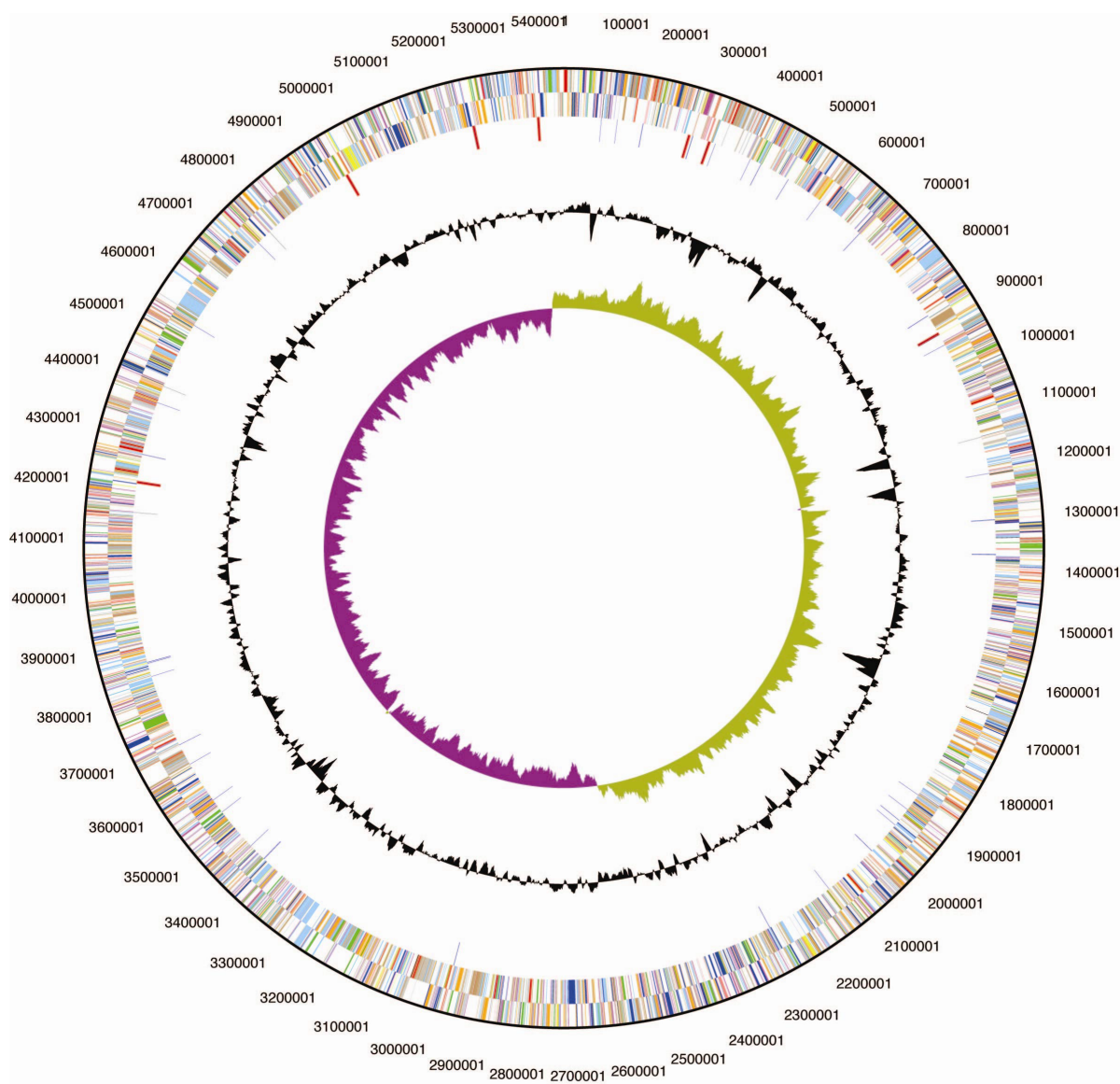


Figure 3. Graphical circular map of the chromosome. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

Table 3. Genome statistics

Attribute	Value	% of total ^a
Genome size (bp)	5,443,009	100.00%
DNA Coding region (bp)	4,772,809	87.69%
DNA G+C content (bp)	3,045,986	55.96%
Total genes ^b	5,139	100.00%
RNA genes	112	2.18%
rRNA operons	7	0.14%
Protein-coding genes	4,952	96.36%
Pseudo genes	75	1.46%
Genes in paralog clusters	2721	52.95%
Genes assigned to COGs	3,808	74.10%
Genes assigned in Pfam domains	4,184	81.41%
Genes with signal peptides	675	13.13%
Genes with transmembrane helices	1,228	23.89%
CRISPR repeats	1	

a) The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

Table 4. Number of genes associated with the 25 general COG functional categories

Code	Value	% age	Description
J	201	4.27	Translation, ribosomal structure and biogenesis
A	1	0.02	RNA processing and modification
K	481	10.22	Transcription
L	160	3.40	Replication, recombination and repair
B	1	0.02	Chromatin structure and dynamics
D	37	0.79	Cell division and chromosome partitioning
Y	0	0.00	Nuclear structure
V	64	1.36	Defense mechanisms
T	187	3.97	Signal transduction mechanisms
M	265	5.63	Cell envelope biogenesis, Outer membrane
N	94	2.00	Cell motility and secretion
Z	0	0.00	Cytoskeleton
W	0	0.00	Extracellular structure
U	116	2.47	Intracellular trafficking and secretion
O	153	3.25	Posttranslational modification, protein turnover, chaperones
C	272	5.78	Energy production and conversion
G	424	9.01	Carbohydrate transport and metabolism
E	470	9.99	Amino acid transport and metabolism
F	106	2.25	Nucleotide transport and metabolism
H	185	3.93	Coenzyme metabolism
I	135	2.87	Lipid metabolism
P	285	6.06	Inorganic ion transport and metabolism
Q	133	2.83	Secondary metabolite biosynthesis, transport and catabolism
R	537	11.41	General function prediction only
S	398	8.46	Function unknown
-	918	17.86	Not in COGs

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