Complete genome sequences of the \textit{Serratia plymuthica} strains 3Rp8 and 3Re4-18, two rhizosphere bacteria with antagonistic activity towards fungal phytopathogens and plant growth promoting abilities

Eveline Adam *, Henry Müller, Armin Erlacher and Gabriele Berg

Abstract

The \textit{Serratia plymuthica} strains 3Rp8 and 3Re4-18 are motile, Gram-negative, non-sporulating bacteria. Strain 3Rp8 was isolated from the rhizosphere of \textit{Brassica napus} L. and strain 3Re4-18 from the endorhiza of \textit{Solanum tuberosum} L. Studies have shown in vitro activity against the soil-borne fungi \textit{Verticillium dahliae} Kleb., \textit{Rhizoctonia solani} Kühn, and \textit{Sclerotinia sclerotiorum}. Here, we announce and describe the complete genome sequence of \textit{S. plymuthica} 3Rp8 consisting of a single circular chromosome of 5.5 Mb that encodes 4954 protein-coding and 108 RNA-only encoding genes and of \textit{S. plymuthica} 3Re4-18 consisting of a single circular chromosome of 5.4 Mb that encodes 4845 protein-coding and 109 RNA-only encoding genes. The whole genome sequences and annotations are available in NCBI under the locus numbers CP012096 and CP012097, respectively. The genome analyses revealed genes putatively responsible for the promising plant growth promoting and biocontrol properties including predicting factors such as secretion systems, iron scavenging siderophores, chitinases, secreted proteases, glucanases and non-ribosomal peptide synthetases, as well as unique genomic islands.

Keywords: \textit{Serratia plymuthica}, Biocontrol, Plant growth promotion, Secretion systems, Antagonistic rhizosphere bacteria

Abbreviations: MIC, Minimal inhibitory concentration; PGP, Plant growth promoting; SMRT, Single molecule, real-time

Introduction

\textit{Serratia} species are well known for their potential as biocontrol agents with broad-spectrum antagonistic activities against common phytopathogens and their plant growth-promoting abilities. \textit{Serratia plymuthica} 3Rp8 was isolated as an indigenous colonizer of oilseed rape (\textit{Brassica napus} L.) rhizosphere and is an in vitro antagonist of the soil-borne fungal phytopathogens \textit{Verticillium dahliae} Kleb., \textit{Rhizoctonia solani} Kühn and \textit{Sclerotinia sclerotiorum} [1] which can cause severe yield losses in a large number of different crops. Chitinase and protease activity were demonstrated by plate assays and the production of N-acylhomoserine lactones was detected using bioluminescent sensor plasmid pSB403 [1, 2]. \textit{Serratia plymuthica} 3Re4-18 was isolated from the endorhiza of a potato plant (\textit{Solanum tuberosum} L.) and was identified as the most effective isolate in an in vitro study screening potato-associated bacterial communities for antagonistic functions against plant pathogenic fungi [3]. Both strains were sequenced to augment current studies targeting novel biotechnological applications for seed and root treatment since the strains represent promising candidates for biological control. In this report, we summarize the complete genome sequences and annotations of \textit{S. plymuthica} 3Rp8 and 3Re4-18 and...
describe their genomic properties. Analysis of the genomes of 3Rp8 and 3Re4-18 will provide a framework for further studies of their rhizosphere competence, biocontrol properties, and plant growth promoting activity. 3Rp8 and 3Re4-18 are deposited in the strain collection of antagonistic microorganisms at Graz University of Technology, Institute of Environmental Biotechnology, Austria.

Organism information

**Classification and features**

*S. plymuthica* 3Rp8 and 3Re4-18 are motile, Gram-negative, non-sporulating *Enterobacteriaceae*. Colonies appear yellow-beige opaque, domed and moderately mucoid with smooth margins on Luria-Bertani (LB) solid media and form colonies within 24 h at 20 °C (Fig. 1a-b). Both strains grow in standard complex media such as LB, potato dextrose agar (PDA), Waksman agar (WA) and nutrient agar (NA) [4] as well as in minimal medium such as Standard Succinate Medium (SSM). The standard growth temperature is at 30 °C, but both strains can replicate in liquid LB at 5 °C and at 40 °C as well. Both strains do not show a production of red pigments on the media mentioned above. The rod-shaped cells are approximately 0.5 μm in width and 2.0 μm in length (Fig. 1c-d).

3Rp8 was isolated from the roots of oilseed rape cultivar Express grown for a field trial in Braunschweig (Germany) in 1998 [1, 5]. 3Re4-18 was isolated from the endorhiza of an early senescent *Solanum tuberosum* L. cultivar Cilena at the experimental station of the Institute for Plant Diseases, Bonn University in Bonn-Poppelsdorf (Germany) in 2001 [3].

Both bacterial strains are efficient colonizer of oilseed rape and cauliflower [4], lettuce and pumpkin roots (unpublished data) and do not cause any obvious negative effects to those hosts. Priming of oilseed rape and cauliflower seeds with the *S. plymuthica* 3Rp8 and 3Re4-18 strains had a significant PGP effect on the root weights of the oilseed rape seedlings [4]. Figure 1e-f shows 3Rp8 and 3Re4-18 colonizing the roots of young lettuce seedlings 1 week after inoculation in a gnotobiotic plant growth approach. The strains have natural resistance to Cefuroxime, Cefuroxime Axetil and Cefoxitin (minimal inhibitory concentration (MIC) > = 64 mg/L) as well as Fosfomycin (MIC > = 256 mg/L). Minimum Information about the Genome Sequences (MIGS) of *S. plymuthica* 3Rp8 and 3Re4-18 are summarized in Table 1, and their phylogenetic position is shown in Figs. 2 and 3. Average nucleotide identity (ANI) data were calculated with Gegenees [6] version 2.2.1 by using a fragmented all against all comparison. The data are illustrated as heat-plot in Fig. 4.
Table 1 Classification and general features of *Serratia plymuthica* 3Rp8 and 3Re4-18 according to the MIGS recommendations [20]

| MIGS ID | Property       | Term                        | Evidence codea |
|---------|----------------|-----------------------------|----------------|
|         | Classification | Domain Bacteria             | TAS [21]       |
|         |                 | Phylum Proteobacteria       | TAS [22]       |
|         |                 | Class Gammaproteobacteria   | TAS [23, 24]   |
|         |                 | Order "Enterobacterales"    | TAS [25]       |
|         |                 | Family Enterobacteriaceae   | TAS [26–28]    |
|         |                 | Genus Serratia              | TAS [26, 29, 30] |
|         |                 | Species Serratia plymuthica | TAS [26, 31]   |
|         | Strain          | *Serratia plymuthica* 3Rp8  | TAS [1]        |
|         |                 | *Serratia plymuthica* 3Re4-18 | TAS [3]    |
|         | Gram stain      | Gram-negative               | TAS [30]       |
|         | Cell shape      | Rod-shaped                  | IDA            |
|         | Motility        | Motile                      | IDA            |
|         | Sporulation     | Non-spore forming           | IDA            |
|         | Temperature     | S-40 °C                     | IDA            |
|         | Optimum         | 30 °C                       | IDA            |
|         | pH range; Optimum | S-9; 6                     | IDA            |
|         | Carbon source   | Heterotrophic               | IDA, TAS [1, 3]

*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 [34].

Genome sequencing information

**Genome project history**

The strains *S. plymuthica* 3Rp8 and 3Re4-18 were selected for sequencing due to their in vitro activity against *V. dahliae* and *R. solani*, their production of hydrolytic enzymes and their root-associated lifestyle on plants [1, 3, 4]. The sequence data will help to reveal genetic features responsible for their plant growth promoting effects and their ability to protect seeds against fungal threats during germination. The genome project is deposited in the NCBI BioProject database under ID 289082 with the Biosample UIDs 3841799 and 3841798, respectively. The finished genome sequences are deposited in GenBank under the accession numbers CP012096 and CP012097, respectively. A summary of the project information is shown in Table 2.

**Growth conditions and genomic DNA preparation**

3Rp8 and 3Re4-18 were grown in 50 ml of nutrient broth II (NB II) (Sifin, Berlin, Germany) medium and incubated for 20 h at 30 °C. 0.5 ml was then centrifuged at 2500 x g for 5 min at 4 °C and genomic DNA was extracted using the MasterPure DNA purification kit (Epicentre, Madison, WI, USA). DNA quality and quantity were checked by agarose gel electrophoresis and spectrophotometry using a UV-Vis spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific, Waltham, MA USA). Total genomic DNA of 3Rp8 (50.7 μg; 0.8 μg μL⁻¹) and of 3Re4-18 (102.8 μg; 1.7 μg μL⁻¹) was sent on dry ice to the sequencing service.

**Genome sequencing and assembly**

PacBio RS libraries with inserts of 8 to 20 kb were constructed and sequenced at GATC Biotech (Konstanz, Germany) using single molecule, real-time (SMRT) sequencing. Assemblies were completed with the Hierarchical Genome Assembly Process v. 2.2.0 (HGAP) algorithm implemented in the PacBio SMRT Analysis software (Pacific Biosciences, Menlo Park, CA, USA). The assembly of the 3Rp8 genome was based on 119,662 quality reads with a mean length of 4581 bp resulting in a single circular chromosome consisting of 5,546,041 bp with 81-fold overall coverage. For assembling the genome of 3Re4-18, 127,834 quality reads with a mean length of 5358 bp were used resulting in a single circular chromosome of 5,439,574 bp with 110-fold overall coverage.

**Genome annotation**

Automatic annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (released 2013). Additional annotation for using the automated assignment of clusters of orthologous groups (COG)-functions to protein-coding genes was completed on
the BASys Web server using Glimmer gene prediction [7–9]. Prediction of Pfam domains, signal peptides and transmembrane helices were calculated using BASys Web Server [7–9], SignalP [10, 11] and TMHMM [12, 13], respectively.

**Genome properties**

The genome of *S. plymuthica* strain 3Rp8 is composed of one circular chromosome consisting of 5,546,041 bp with an average GC content of 56.07 % (Table 3 and Fig. 5a). Among the 5130 predicted genes, 4954 (96.57 %) were identified as protein coding genes, 68 (1.33 %) were designated as pseudo genes, 22 (0.43 %) as rRNAs, 85 (1.66 %) as tRNAs and one (0.02 %) as ncRNA. 21 (0.41 %) genes were frameshifted.

The genome of *S. plymuthica* strain 3Re4-18 is composed of one circular chromosome of 5,439,574 bp with an average GC content of 56.24 % (Table 3 and Fig. 5b).
Among the 5005 predicted genes, 4845 (96.80 %) were identified as protein coding genes, 51 (1.02 %) were designated as pseudo genes, 22 (0.44 %) as rRNAs, 86 (1.72 %) as tRNAs and one (0.02 %) as ncRNA. 19 (0.38 %) genes were frameshifted.

The GC contents of both strains are similar to that of other S. plymuthica strains. The classification of CDSs into functional categories according to the COG database [14, 15] is summarized in Table 4 on BASys gene prediction.

Table 2 Project information

| MIGS ID | Property Term |
|---------|---------------|
| MIGS 31 | Finishing quality | Finished |
| MIGS 28 | Libraries used | PacBio RS libraries with inserts of 8 to 20 kb |
| MIGS 29 | Sequencing platforms | PacBio RS II |
| MIGS 31.2 | Fold coverage | 3Rp8 - 81 x 3Re4-18 - 110 x |
| MIGS 30 | Assemblers | Celera Assembler + Hierarchical genome assembly process v. 2.2.0 |
| MIGS 32 | Gene calling method | NCBI Prokaryotic Genome Annotation Pipeline, Glimmer gene prediction |

Locus Tag | 3Rp8 - ADP72, 3Re4-18 - ADP73 |
| Genbank ID | 3Rp8 - CP012096, 3Re4-18 - CP012097 |
| GenBank Date of Release | June 15, 2016 |
| GOLD ID | 3Rp8 - Gp0137066, 3Re4-18 - Gp0131532 |
| BIOPROJECT | PRJNA289082 |
| MIGS 13 | Source Material Identifier | 3Rp8 - SAMN03841799, 3Re4-18 - SAMN03841798 |
| Project relevance | Agricultural, Environmental |

Insights from the genome sequence

Both strains share a collection of genes that may be important contributors to biological control with other S. plymuthica strains already published, like genes annotated as secretion systems, iron scavenging siderophores ( locus tags ADP72_19185, ADP73_16995 ), chitinases ( e.g. locus tags ADP72_04805, ADP73_00825 ), secreted proteases ( e.g. locus tags ADP72_11930, ADP73_24375 ), glucanases ( e.g. locus tags ADP72_10355, ADP73_00890 ) and non-ribosomal peptide synthetases ( e.g. locus tags ADP72_05100, ADP73_05800 ). Additionally, genes predicting plant growth promotion, like spermidine synthases ( e.g. locus tags ADP72_15170, ADP73_11985 ), indole-3-pyruvate decarboxylases ( locus tags ADP72_18190, ADP73_17980 ) or diacetyl-reductase ( locus tags ADP72_19475, ADP73_16745 ) were detected. Unique genomic islands were identified in both strains with IslandViewer 3 software [16–18]. In 3Rp8 coding regions containing high similarities on DNA-level with a region in Photorhabdus luminescens TT01 [19] as well as a region annotated as type IV/VI secretion system were found. In 3Re4-18 unique coding regions for proteins related to type VI secretion systems as well as other islands with putatively phage origin were detected.

Conclusions

Here, we announce the complete genome sequences of Serratia plymuthica 3Rp8 and 3Re4-18, two endobacteria that were originally isolated in Germany from oilseed rape rhizosphere and from endorhiza of potato, respectively. Both strains were selected for sequencing based on their ability to control soil-borne plant-pathogenic fungi. Such properties likely have origins in a repertoire of genes probably involved in fungal cell wall degradation expressed by chitinases, proteases or non-ribosomal peptide synthetases. They also share a
Table 3 Genome statistics

| Attribute                        | 3Rp8            | % of Total | 3Re4-18    | % of Total |
|----------------------------------|-----------------|------------|------------|------------|
| Genome size (bp)                 | 5,546,041       | 100.00     | 5,439,574  | 100.00     |
| DNA coding (bp)                  | 4,745,098       | 85.56      | 4,683,982  | 86.11      |
| DNA G+C (bp)                     | 3,109,696       | 56.07      | 3,058,992  | 56.24      |
| DNA scaffolds                     | 1               | -          | 1          | -          |
| Total genes                      | 5130            | 100.00     | 5005       | 100.00     |
| Protein coding genes             | 4954            | 96.57      | 4845       | 96.80      |
| RNA genes                        | 108             | 2.11       | 109        | 2.18       |
| Pseudo genes                     | 68              | 1.33       | 51         | 1.02       |
| Genes in internal clusters       | NA              | -          | NA         | -          |
| Genes with function prediction   | 4278            | 83.39      | 4239       | 84.70      |
| Genes assigned to COGs           | 4077            | 79.47      | 4017       | 80.26      |
| Genes with Pfam domains          | 3829            | 74.64      | 3780       | 75.52      |
| Genes with signal peptides       | 499             | 9.73       | 489        | 9.77       |
| Genes with transmembrane helices | 1239            | 24.15      | 1213       | 24.24      |
| CRISPR repeats                   | 0               | 0          | 0          | 0          |

*The total is based on either the size of the genome in base pairs or the total number of genes in the annotated genome.

Fig. 5 Graphical map of the chromosome of 3Rp8 (a) and 3Re4-18 (b). The outer scale is marked every 10 kb. Circles range from 1 (outer circle) to 7 (inner circle). Circle 1 and 2, ORFs encoded by leading and lagging strand respectively, with color code for functions: salmon, translation, ribosomal structure and biogenesis; aquamarine, RNA processing and modification; light blue, transcription; cyan, DNA replication, recombination and repair; tan, chromatin structure and dynamics; turquoise, cell division; dark orange, defense mechanisms; deep pink, post-translational modification, protein turnover and chaperones; dark olive green, cell envelope biogenesis; purple, cell motility and secretion; lavender, intracellular trafficking, secretion, and vesicular transport; forest green, inorganic ion transport and metabolism; pink, signal transduction; red, energy production; sienna, carbohydrate transport and metabolism; yellow, amino acid transport; orange, nucleotide transport and metabolism; gold, co-enzyme transport and metabolism; cornflower blue, lipid metabolism; blue, secondary metabolites, transport and catabolism; gray, general function prediction only; yellow green, unknown function; black, function unclassified or unknown. Circle 3 and 4, distributions of tRNA genes and rrn operons respectively. Circle 5, distribution of pseudogenes. Circle 6 and 7, G+C content and GC skew (G-C/G+C) respectively.
collection of genes known to be responsible for specific PGP features and both carry unique genomic islands with interesting genes for agricultural applications. Further functional studies and comparative genomics with related isolates will greatly enhance the understanding of biocontrol and PGP features.

Acknowledgements
The authors thank NAWI Graz for providing technical devices for confocal laser scanning microscopy, Kathrin Hölzl (Graz) for antibiotic resistance characterization of the strains, John Hunter Allan (Dundee) for growth experiments and Christin Zachow (Graz) for proofreading of the manuscript.

Funding
This Project was supported by grant id 836466 (FFG project “Novel biotechnological processes for seed and root applications”) from the Austrian Research Promotion Agency to GB, that was co-funded by Biotenzz GmbH.

Table 4 Number of genes associated with general COG functional categories

| Code | 3Rp8 Value | 3Re4-18 Value | Description |
|------|------------|---------------|-------------|
| J    | 169 2.90   | 167 2.97      | Translation, ribosomal structure and biogenesis |
| A    | 1 0.02     | 1 0.02        | RNA processing and modification |
| K    | 441 7.57   | 445 7.92      | Transcription |
| L    | 170 2.92   | 152 2.70      | Replication, recombination and repair |
| B    | 1 0.02     | 1 0.02        | Chromatin structure and dynamics |
| D    | 27 0.46    | 28 0.50       | Cell cycle control, cell division, chromosome partitioning |
| V    | 58 1.00    | 56 1.00       | Defense mechanisms |
| T    | 141 2.42   | 146 2.60      | Signal transduction mechanisms |
| M    | 256 4.40   | 256 4.55      | Cell wall/membrane biogenesis |
| N    | 99 1.70    | 90 1.60       | Cell motility |
| U    | 54 0.93    | 49 0.92       | Intracellular trafficking and secretion |
| O    | 153 2.63   | 148 2.60      | Posttranslational modification, protein turnover, chaperones |
| C    | 261 4.48   | 259 4.61      | Energy production and conversion |
| G    | 412 7.08   | 406 7.22      | Carbohydrate transport and metabolism |
| E    | 442 7.59   | 433 7.70      | Amino acid transport and metabolism |
| F    | 89 1.53    | 90 1.60       | Nucleotide transport and metabolism |
| H    | 144 2.47   | 145 2.58      | Coenzyme transport and metabolism |
| I    | 150 2.58   | 158 2.45      | Lipid transport and metabolism |
| P    | 246 4.22   | 246 4.38      | Inorganic ion transport and metabolism |
| Q    | 96 1.65    | 91 1.62       | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 383 6.58   | 385 6.85      | General function prediction only |
| S    | 284 4.88   | 285 5.07      | Function unknown |
| -    | 1746 29.98 | 1605 28.55    | Not in COGs |

The percentage is based on the total number of protein coding genes in the genome based on BASys gene prediction [7–9]

Authors’ contributions
EA wrote the manuscript, participated in the design of the study and performed the statistical analysis and annotation. HM conceived the study, participated in its design and coordination, and carried out the molecular genetic experiments and the sequence alignment. AE provided the photographs and microscopic images. EA, HM, AE and GB commented on the manuscript at all stages. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 18 June 2016 Accepted: 27 August 2016
Published online: 06 September 2016

References
1. Berg G, Roskot N, Steidle A, Eberl L, Zock A, Smalla K. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different Verticillium host plants. Appl Environ Microbiol. 2002;68(7):3328–38.
2. Winston MK, Swift S, Fish L, Throup JP, Jørgensen F, Ram S. Construction and analysis of luxCDABE-based plasmid sensors for investigating N-acyl homoserine lactone-mediated quorum sensing. FEMS Microbiol Lett. 1998;163:85–92.
3. Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, Hallmann J. Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol Ecol. 2005;51(2):215–29.
4. Rybakova D, Schnurr M, Wetzlinger U, Vareo-Sarez A, Murgu O, Müller H, et al. Kill or cure? The interaction between endophytic Pannibacillus and Senataxis strains and the host plant is shaped by plant growth conditions. Plant Soil. 2015. doi:10.1007/s11104-015-2572-z.
5. Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, et al. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol. 2001;67(10):4742–51.
6. Ågren J, Sundström A, Höfström T, Segerman B. Genegesee: fragmented alignments of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. PLoS One. 2012;7(6):e39107.
7. Van Domselaar GH, Stothard P, Shrivastava S, Cruz JA, Guo A, Dong X, et al. BASys: a web server for automated bacterial genome annotation. Nucleic Acids Res. 2005;33:W455–9.
8. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. Improved microbial gene identification with GLIMMER. Nucleic Acids Res. 1999;33:W455–9.
9. Smallberg SL, Delcher AL, Kasif S, White O. Microbial gene identification using interpolated Markov models. Nucleic Acids Res. 1998;26:544–8.
10. Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods. 2011;8(10):785–91.
11. Emanuelsson O, Brunak S, von Heijne G, Nielsen H. Locating proteins in the cell using TargetP, SignalP and related tools. Nat Protoc. 2007;2:953–71.
12. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol. 2001;305:567–78.
13. TMHMM Server v. 2.0. Prediction of transmembrane helices in proteins. Center for Biological Sequence Analysis. Technical University of Denmark DTU, Lyngby. 2015. www.cbs.dtu.dk/services/TMHMM/. Accessed 22 Feb 2016.
14. Tatusov RL, Koonin EV, Lipman DJ. A genomic perspective on protein families. Science. 1997;278:631–7.
15. Clusters of Orthologous Groups. NCBI. 2015. http://www.ncbi.nlm.nih.gov/COG. Accessed 26 Feb 2016.
16. Dhillon B, Chiu T, Laird M, Langille M, Brinkman F. IslandViewer update: improved genomic island discovery and visualization. Nucleic Acids Res. 2013;41(W1):129–32.
17. Dhillon B, Laird M, Shie J, Winsor G, Lo R, Nizam F, et al. IslandViewer 3: more flexible, interactive genomic island discovery, visualization and analysis. Nucleic Acids Res. 2015;43(1):W104–8.
18. Langille M, Brinkman F. IslandViewer: an integrated interface for computational identification and visualization of genomic islands. Bioinformatics. 2009;25(S):i664–5.
19. Duchaud E, Rusniok C, Frangeul L, Buchrieser C, Givaudan A, Taourit S, et al. The genome sequence of the entomopathogenic bacterium *Photorhabdus luminescens*. Nat Biotechnol. 2003;21:1307–13.

20. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, et al. The minimum information about a genome sequence (MIGS) specification. Nat Biotechnol. 2008;26:541–7.

21. Woese CR. Towards a natural system of organisms: Proposal for the domains *Archea*, *Bacteria* and *Eucarya*. Proc Natl Acad Sci U S A. 1990;87:4576–9.

22. Garrity GM, Bell JA, Lilburn T. Phylum XIV. *Proteobacteria* phyl. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. *Bergey's Manual of Systematic Bacteriology*, 2nd ed, Volume 2, Part B. New York: Springer; 2005. p. 1.

23. Garrity GM, Bell JA, Lilburn T. Class III. *Gammaproteobacteria* class. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. *Bergey's Manual of Systematic Bacteriology*, 2nd ed, Volume 2, Part B. New York: Springer; 2005. p. 587.

24. Validation of publication of new names and new combinations previously effectively published outside the IJSEM. List no. 106. Int J Syst Evol Microbiol. 2005;55:225–8.

25. Garrity GM, Holt JG. Taxonomic outline of the *Archea* and *Bacteria*. In: Garrity GM, Boone DR, Castenholz RW, editors. *Bergey’s Manual of Systematic Bacteriology*, vol. 1. 2nd ed. New York: Springer; 2001. p. 155–66.

26. Skerman VBD, McGowan V, Sneath PHA. Approved lists of bacterial names. Int J Syst Bacteriol. 1980;30:225–420.

27. Brenner DJ, Farmer JJ. Family I. *Enterobacteriaceae* Rahn 1937. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. *Bergey’s Manual of Systematic Bacteriology*, Volume 2, Part B. New York: Springer; 2005. p. 587.

28. Rahn O. New principles for the classification of bacteria. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abteilung II. 1937;96:273–86.

29. Bizio B. Lettera di Bartolomeo Bizio al chiarissimo canonico Angelo Bellani sopra il fenomeno della polenta porporina. Biblioteca Italiana o sia Giornale di Letteratura. [Anno VIII]. Scienze e Arti. 1823;30:275–95.

30. Grimont F. Genus GPAD, XXXIV. *Serratia* Bizio 1823, 288AL. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. *Bergey’s Manual of Determinative Bacteriology*, 6th ed. Baltimore: Williams and Wilkins Co; 1948. p. 481–2.

31. Neupane S, Högborg N, Alström S, Lucas S, Han J, Lapidus A, et al. Complete genome sequence of the rapeseed plant-growth promoting *Serratia plymuthica* strain AS9. Stand Genomic Sci. 2012;6:54–62.

32. Zachow C, Pirker H, Westendorf C, Tilcher R, Berg G. The *Caenorhabditis elegans* assay: a tool to evaluate the pathogenic potential of bacterial biocontrol agents. Eur J Plant Pathol. 2009;125(3):367–76.

33. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene Ontology: tool for the unification of biology. Nat Genet. 2000;25:25–9.

34. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016. in press.