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DATA NOTE

Genome of *Serratia plymuthica* UBCF_13, Insight into diverse unique traits

[version 1; peer review: 1 not approved]

Raudhatul Fatiah¹, Irfan Suliansyah^{2,3}, Djong Hon Tjong^{3,4}, Lily Syukriani², Roza Yunita², Robi Trivano², Nurefni Azizah², Jamsari Jamsari^{1,3}

¹Agricultural Science, Universitas Andalas, Padang, West Sumatera, 25163, Indonesia²Agrotechnology, Agricultural Faculty, Universitas Andalas, Padang, West Sumatera, 25163, Indonesia³Biotechnology Magister Program, Universitas Andalas, Padang, West Sumatera, 25163, Indonesia⁴Biology, Mathematics and Life Sciences Faculty, Universitas Andalas, Padang, West Sumatera, 25163, Indonesia

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Abstract

Background: Whole genome sequencing is become an essential tool to explore potential of microorganism and evolutionary study. The *Serratia plymuthica* UBCF_13 is one of phylloplane associated plant bacteria showing antifungal activity. For that reason, its complete genome information is necessary to enhance its potential as biocontrol against plant pathogenic fungal. Here, we report the genome sequence of *Serratia plymuthica* UBCF_13 to understand the molecular mechanism regarding its biocontrol ability.

Methods: Continuous short reads were attained from Illumina sequencing runs and reads 150 bp were merged into a single dataset. Pan-genome based method was used to identify core-genome of *S. plymuthica* species and unique gene in UBCF_13.

Results: Assambled Illumina reads of *S. plymuthica* strain UBCF_13 genome was produced a 5.46 Mb circular genome sequence. It was found 3321 genes belong to the core-genome sheared by the 18 strains evaluated. The UBCF_13 genome harbor 485 unique genes, where 300 of them only can be found in this strain

Conclusions: The sequence of UBCF_13 genome sequence data will contribute for further exploration of the potential of *S. plymuthica* UBCF_13 as bacteria producing antibiotic.

Keywords

Pan-genomic, core-genome, unique gene, *Serratia plymuthica*, genome sequencing, comparative genomic

Open Peer Review

Approval Status

1

version 1

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view

1. Zulema Udaondo , University of Arkansas for Medical Sciences, Little Rock, USA

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Jamsari Jamsari (jamsari@agr.unand.ac.id)

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Introduction

Serratia plymuthica bacteria have been isolated from many environmental sources and are found associated with diverse plants^{1–5}. Many strains of this species have been reported to have the ability to inhibit the growth of plant-pathogenic fungi and stimulate plant growth^{6–9}. UBCF_13 is one strain of this species. It has ability to inhibit *Colletotrichum gloeosporioides*, a species of post-harvest pathogenic fungi that causes anthracnose disease in various plants¹⁰.

Here, we report the complete genome sequence of this bacterium, constructed using Illumina sequencing technology. Our dataset may be useful as a comparative genome for evolutionary and speciation studies, as well as for the analysis of protein-coding RNA, biosynthetic gene clusters and may also useful for further study such as the regulation of gene expression in relation to the antifungal activity of this bacterium.

Methods

Genomic DNA isolation and sequencing

S. plymuthica strain UBCF_13 was isolated from phylloplane of *Brassica juncea* L. in 2012 from District of Solok, Province of West Sumatera, Indonesia¹⁰. The bacterium was cultivated in Luria–Bertani (LB) broth at 27°C for 16 hours with 150 rpm. The genomic DNA was extracted using the method of Chen and Kuo (1993)¹¹, followed by degrading residual RNA by RNase. Library preparation and sequencing was done by Novogen (Hong Kong). Sequencing was performed using Illumina NovaSeq 6000 (Illumina NovaSeq 6000 Sequencing System, RRID:SCR_016387).

Genome assembly and annotation

Continuous short reads of 150 bp were merged into a single dataset. The dataset was obtained by using combination of map-based gene references and de novo assembly that was performed in Geneious software (Geneious, RRID:SCR_010519)¹². The annotation in genome submission was carried out using NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAP)¹³. The annotated genome sequence of UBCF_13 has been deposited in the NCBI GenBank under accession number CP068771.

Comparative genomics of *Serratia plymuthica* strains

Comparative genomics analysis was carried out using genome sequences of UBCF_13 from this research and 17 whole sequenced genomes of other *Serratia plymuthica* strains retrieved from NCBI's GenBank. The genomes were reannotated using the Prokka software tool (Prokka, Galaxy Version 1.14.6+galaxy0) (Prokka, RRID:SCR_014732)^{14,15} that is available from the NCBI. Identification of genes shared between the strains, and 'presence-absence gene set' was carried

out using Roary; Galaxy Version 3.13.0+galaxy1, (Roary, RRID:SCR_018172)¹⁶ with a threshold similarity of 70%.

Genes that exist in all the strains are the core-genome. Phylogenetic trees were constructed using Maximum Likelihood based inference of large phylogenetic trees-RAXML, Galaxy Version 8.2.4+galaxy2 (RAXML, RRID:SCR_006086)¹⁷ based on multialignment of concatenate core-genome. Phandango (Phandango, RRID:SCR_015243)¹⁸ was used to view the resulted output graphs.

Cluster of the orthologous groups of UBCF_13

The translated protein coding genes of UBCF_13 was used for identification of cluster of orthologous groups (COG). This was obtained from NCBI BLAST+rpsblast (Galaxy Version 2.10.1+galaxy0)¹⁹ and eggNOG Mapper (Galaxy Version 2.0.1+galaxy1) (eggNOG, RRID:SCR_002456)²⁰. The result of COG identification was classified based on the categories in COG database NCBI²¹.

Result and discussion

Comparative genomics of *Serratia plymuthica* strains

The whole genome sequencing reads of *Serratia plymuthica* UBCF_13 were assembled into a single circular 5.46 Mb chromosome with overall GC content of 56.2% (Table 1). *S. plymuthica* has a genome size in the range 5.40–5.70 Mb. The GC content percentage is 55.70–56.60. Based on genome reannotation by Prokka, it was found different number of CDS in each genome *S. plymuthica* (Table 1). All of the compared *S. plymuthica* genomes shared a highly conserved genomic architecture as inferred from synteny of protein coding orthologs.

Figure 1A shows the phylogenetic tree of 18 strains *S. plymuthica*. The phylogenetic tree shows that *S. plymuthica* UBCF_13 is in same cluster with strain AS9, PRI-2C, NCTC8015, and NCTC8900. The strain PRI-2C is reclassified and transferred to the species *S. inhibens*²². The pangenome was performed together with other strains in order to obtain further insight into specific features in the UBCF_13. It was found 3315 belong to the core-genome shared by the 18 strains evaluated. The genome of the UBCF_13 harbors 488 unique genes, of which 300 genes are only contained by this strain. The presence-absence gene set was shown in file supplementary data 1

The Cluster of Orthologous Groups of UBCF_13

Functional categories of the CDS in *S. plymuthica* UBCF_13 based on the Cluster of Orthologous Groups (COG) categories are shown in Table 2. The list of UBCF_13 COG and its function classification based on COG database was shown in extended dataset 2²³.

Table 1. Genomic features comparison of *Serratia plymuthica* strains.

Strains	Accessions	Size (Mb)	GC%	CDSs	Sources
UBCF_13	CP068771.1	5.46	56.20	4920	Phylloplane of <i>Brassica juncea</i> L.
3Re4-18	CP012097.1	5.44	56.20	4937	Endorhiza of <i>Solanum tuberosum</i> L. cv. <i>Cilena</i>
3Rp8	CP012096.1	5.55	56.10	5071	<i>Brassica napus</i>
4Rx13	CP006250.1	5.40	56.09	4831	The rhizosphere of <i>Brassica napus</i>
AS9	CP002773.1	5.44	56.00	4970	Rapeseed roots grown in Sweden
C-1	CP053398.1	5.70	56.10	5316	<i>Capsicum annum</i>
FDAARGOS_889	CP065699.1	5.44	56.20	4951	
FDAARGOS_895	CP065688.1	5.35	55.90	4856	
FDAARGOS_896	CP065747.1	5.53	55.80	5025	
FDAARGOS_907	CP065673.1	5.47	55.90	4964	
FDAARGOS_1138	CP068096.1	5.48	56.17	4960	
NCTC12961	LS483469.1	5.35	55.90	5833	
NCTC8015	LR134478.1	5.33	55.70	4901	
NCTC8900	LR134151.1	5.32	55.80	5148	
PRI-2c	CP015613.1	5.47	55.70	5009	Maize rhizosphere in the Netherlands
S13	CP006566.1	5.47	56.20	4959	Raw vegetable-processing line
RVH1	ARWD01000001.1	5.51	56.20	5023	
V4	CP007439.1	5.51	56.20	5073	A biofilm of pasteurizer plates of milk processing machine

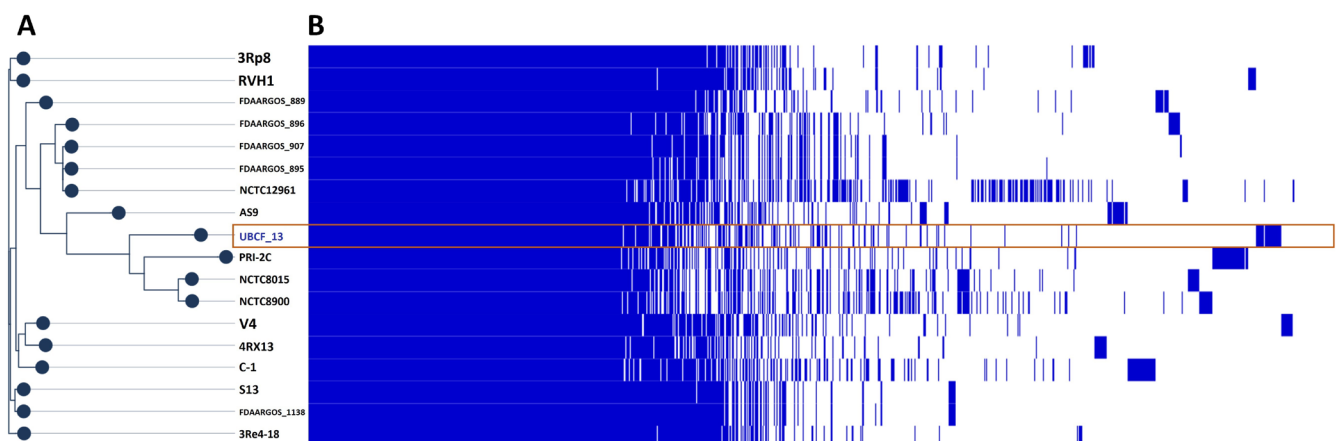


Figure 1. Maximum Likelihood tree and visualization of comparative genomic (pan genomic) between 18 strain *S. plymuthica*. (a) Phylogenetic of 18 strains *S. plymuthica* based on concatenate core-genome multialignment; and (b) visualization of presence (blue bar)-absence (white bar) gene in each of the strains.

Table 2. Cluster of Orthologous Gene of The UBCF_13.

	Function Class	Number of genes
A	RNA processing and modification	1
C	Energy production and conversion	259
D	Cell cycle control, cell division, chromosome partitioning	61
E	Amino acid transport and metabolism	583
F	Nucleotide transport and metabolism	120
G	Carbohydrate transport and metabolism	476
H	Coenzyme transport and metabolism	255
I	Lipid transport and metabolism	215
J	Translation, ribosomal structure and biogenesis	272
K	Transcription	526
L	Replication, recombination and repair	153
M	Cell wall/membrane/envelope biogenesis	311
N	Cell motility	96
O	Posttranslational modification, protein turnover, chaperones	212
P	Inorganic ion transport and metabolism	367
Q	Secondary metabolites biosynthesis, transport and catabolism	125
R	General function prediction only	476
S	Function unknown	178
T	Signal transduction mechanisms	233
U	Intracellular trafficking, secretion, and vesicular transport	82
V	Defense mechanisms	131
W	Extracellular structures	30
X	Mobilome: prophages, transposons	42
Z	Cytoskeleton	2
-	No COG assignment	518

Data availability

Data from *Serratia plymuthica* UBCF_13 is available at NCBI under Bio-Project [PRJNA692765](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA692765), including the complete genome with annotation at GenBank accession [CP068771](https://www.ncbi.nlm.nih.gov/nuccore/CP068771), and the read data in the Sequence Read Archive (SRA) database under the accession number SRR15012717.

Extended data

Dataset 1:

Dryad: Gene presence absence in *Serratia plymuthica* strains. <https://doi.org/10.5061/dryad.1zcrjdfsj>²⁴

This project contains the following extended data;

- Gene_presence_absence_in_Serratia_plymuthica_strains.csv (This data shows gene presence or absence

across strain *Serratia plymuthica* species. It contains a list of coding protein gene (CDS) name and region where the gene exist in each genome (locus_tag). The region/locus where the CDS are existing has a specific tag for each genome, whereas the number after locus names represents the order of CDS in each genome. This data could be used to identify conserved and unique genes in *Serratia plymuthica*.)

- Readme_Gene_presence_absence_in_Serratia_plymuthica_strains.txt (This text file provides information about the above data)

Dataset 2:

Dryad: Classification of the UBCF_13 COG based on COG database in NCBI <https://doi.org/10.5061/dryad.sn02v6x4g>²³

This project contains the following extended data;

- Classification_of_the_UBCF_13_COG_based_on_COG_database_in_NCBI.csv (This data contains information about the assignment of Clusters of Orthologous Gene (COG) for each coding protein gene

in *Serratia plymuthica* UBCF_13 and their functional classification based on COG database in NCBI)

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

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<http://www.doi.org/10.5061/dryad.1zcrjdfj>

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Zulema Udaondo 

Department of Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, 72205, USA

The manuscript by Fatiah *et al.* is a descriptive study of the novel bacteria with antifungal activity, *Serratia plymuthica* UBCF_13 that was isolated from the leaf surface of *Brassica juncea*. The pangenome of this isolate along with another 17 *Serratia plymuthica* strains downloaded from GenBank was also performed by the authors.

S. plymuthica is a cosmopolite species that have potential applications as a biocontrol agent due to its antifungal activity. Therefore comparative analyses of members of this species are useful to unveil the metabolic characteristics of this interesting microorganism.

Although the manuscript is understandable for the most part, I would strongly recommend a further revision of the manuscript by a scientific editor as there are some sentences in the manuscript that are difficult to understand, hindering the readability of the entire manuscript.

There are also some important data and methodology that are lacking in the manuscript that would be useful from my point of view to better understand the complete context of the study and the reported findings.

For example, one of the main concerns is that when accessing the GenBank accession ID for the UBCF_13 strains provided by the authors, it can be observed in the taxonomy check performed by GenBank staff, that this strain is flagged as "Inconclusive". Thus, when checking the bottom of the Assembly QA tab from the GenBank webpage:

https://www.ncbi.nlm.nih.gov/assembly/GCF_018336935.1#/qa, this strain is classified as a member of the species *Serratia inhibens*.

This species is also the one to which the strain PRI-2C was reclassified in 2020 as can be observed in the paper published in the International Journal of Systematic and Evolutionary Microbiology June 2020¹. Thus, it makes sense that these two strains are grouped together in the tree shown by

the authors. The isolates NCTC8900 and NCTC8015 also failed their taxonomy check, being identified as well, as members of *Serratia inhibens* species, thus it makes sense that these four strains form a monophyletic group in the tree. These are important issues that I believe should be addressed by the authors. Also, this information could help clarify some of the information obtained by the authors in their pangenome analysis.

On the basis of the above, I think it is crucial to provide in the methodology how isolate UBCF_13 was identified as a member of *S. plymuthica* by the authors.

Another issue that I believe would help the authors increase the confidence of their analysis is to provide a quick explanation about the selection of the strains employed in their pangenome analysis. So far there are 29 available genomes classified as *S. plymuthica* in GenBank, however, the authors only utilized 18 strains (taking into account UBCF_13). It would be interesting to know what was the selection method of these strains performed by the authors.

Also, very importantly the sequence similarity cutoff for the pangenome analysis was provided (70%) but not the sequence coverage which is crucial to understand how strict the clustering performed was in the pangenome analysis. Also, it would interesting to know why these cut-offs were selected.

Finally, a brief paragraph summarizing the conclusions obtained by the author after the result section would help wrap up the whole manuscript.

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Is the rationale for creating the dataset(s) clearly described?

No

Are the protocols appropriate and is the work technically sound?

No

Are sufficient details of methods and materials provided to allow replication by others?

No

Are the datasets clearly presented in a useable and accessible format?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Comparative genomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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