# Whole-genome sequences of eight strains of the *Ralstonia solanacearum* species complex associated with bacterial wilt disease in sub-Saharan Africa.

Mary N. Mwangi[1,2], Eric Magembe[1], Kalpana Sharma[1], Gilles Cellier[3], Stephanie Javegny[4], Marc Ghislain[1], Jonathan D G Jones[2]

[1]International Potato Center, P.O. Box 25171, Nairobi 00603, Kenya.

[2]The Sainsbury Laboratory, Norwich Research Park, Norwich, Norfolk, NR4 7UH, UK

[3]Anses – Plant Health Laboratory, Tropical Pests and Diseases unit, Saint Pierre, Reunion Island, France.

[4]CIRAD, UMR Peuplements végétaux et bioagresseurs en milieu tropical, Saint Pierre, Reunion Island, France.

Corresponding authors : *mary.mwangi@cgiar.org, marynmwangi98@gmail.com; **jonathan.jones@TSL.ac.uk

## Abstract

Bacterial wilt, caused by the *Ralstonia solanacearum* species complex (RSSC), is one of the most destructive diseases of potato in sub-tropical regions. This study reports the whole-genome shotgun sequences of eight RSSC strains, isolated from potato (*Solanum tuberosum L.*), *Pelargonium*, *Capsicum annuum*, *Nicotiana tabacum*, symptomatic for bacterial wilt in Sub-Saharan Africa. Sequencing was done on the Illumina NovaSeq 6000 and genomic sequences were deposited in NCBI GenBank under the BioProject PRJNA1070535. *R. solanacearum* strains were assembled into between 84 and 147 contigs with total genome sizes of between 5.23 Mb and 5.62 Mb in length and GC content between 66.49% and 67.08%. These data will provide a useful resource for future studies into RSSC and associated diseases of important crop plants.

## Introduction

The *Ralstonia solanacearum* species complex is composed of soil-borne bacteria and causal agent of the bacterial wilt disease. It is amongst the top ten most destructive phytopathogens (Mansfield et al., 2012). RSSC strains can cause disease in over 300 plant species (De Boer, 2006; Genin, 2010). Bacterial wilt disease of major crop plants including tomato, pepper, potato and the *Solanaceae* family as whole (Kaguongo et al., 2010) leads to significant yield losses across sub-tropical regions each year.

Potato is an important food crop in many sub-Saharan African countries (Kwambai et al., 2023). Along with late blight caused by the oomycete *Phytophthora infestans*, bacterial wilt is a major constraint to potato production in tropical and sub-tropical regions(Priou et al., n.d.) (Priou et al., 1999)(Okeyo et al., 2022). The diversity of *R.solanacearum* species, coupled with its wide host-range and its persistence in the soil are the most significant impediments to the existing control methods (Salanoubat et al., 2002). The aim of this study was to generate whole-genome sequences of eight previously isolated *R. solanacearum* strains identified on plants exhibiting bacterial wilt symptoms, growing in sub-Saharan Africa. The genome data will provide useful genetic information to assist research into this destructive plant pathogen.

## Material and Methods

### *Bacterial isolates and culture*

Eight RSSC strains were isolated from different host plants potato*, Pelargonium*, *Capsicum annuum*, *Nicotiana tabacum*, symptomatic for bacterial wilt and stored as cultures in the CIRAD repository (UMR PVBMT, Reunion Island, France). Metadata summary of eight RSSC strains isolated from diseased host plants symptomatic of bacterial wilt in sub-Saharan Africa as listed in table 1.

### *DNA extraction*

A single colony was inoculated into CPG broth and incubated until turbidity. Cells were pelleted at 3000 *g* for 10 min, resuspended in 0.8 ml CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl (pH8), 1.4 M NaCl, 0.2% β-mercaptoethanol and 0.1 mg/ml proteinase K) and incubated with occasional

inversion at 60°C for 1 h. For phase separation, 0.8 ml chloroform/isoamylalcohol (24:1) was added, the solution mixed by inversion for 2 min and centrifuged at 4000 *g* for 10 min at 4°C. Ribonuclease A (RNase A; Thermo Scientific, UK) was added to the aqueous phase at a final concentration of 10 µl/ml, mixed by inversion and incubated for 30 min.

*Sequencing of bacterial genomic DNA*

DNA was prepared following MicrobesNG DNA submission protocols. Subsequent library preparation, DNA sequencing and bioinformatics was conducted using MicrobesNG, following in-house protocols. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR automated liquid handling system (Hamilton Bonaduz AG, Switzerland). Genomic DNA libraries were prepared using the Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's instructions with the following modifications: input DNA was increased 2-fold, and PCR elongation time increased to 45 seconds. Libraries were sequenced using the lllumina NovaSeq 6000 (Illumina, San Diego, USA) using a 250 bp paired-end protocol.

Reads were adapter trimmed using Trimmomatic version 0.30 (Bolger et al., 2014) with a sliding window quality cutoff of Q15. *De novo* assembly was performed on samples using SPAdes version 3.7 (Bankevich et al., 2012) and the genome contigs annotated with Prokka 1.11 (https://github.com/tseemann/prokka) (Seemann, 2014). The taxonomic labels identification of the genome sequence was achieved by Kraken.

## Results

The sequence reads assembled by Quast revealed a number of contigs ranging from 84 to 147 with the largest contigs of 567972 bp. Genome assemblies were between 5.23 Mb and 5.62 Mb in length with GC content between 66.49% and 67.08%. The results calculated using the taxonomic sequence classifier software Kraken (Wood & Salzberg, 2014) revealed an identity over 98 % of the 8 submitted genome sequences to *Burkholderiaceae* family, over 98% to *Ralstonia* genus and over 92% to *Ralstonia solanacearum* species complex.

Trimmed sequence read data and genome assembly data of the eight *R. solanacearum* strains has been deposited in NCBI GenBank under the BioProject PRJNA1070535. A summary of the genomic features for the eight strains is listed in Table 2.

## Data availability

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession XXXXXX000000000.Raw sequence reads have been deposited in the NCBI Sequence Read Archive under Bioproject number PRJNA1070535 and accessions generated listed in table 2.

**Table 1: Metadata summary of eight *Ralstonia solanacearum* species complex strains isolated from symptomatic host plants in sub-Saharan Africa.**

| CIRAD id | Sample id | COUNTRY | Host | Date of observation | Species strain | Phylotype | Sequevar |
|---|---|---|---|---|---|---|---|
| RUN101 | Capsicum_KE101 | KENYA | *Capsicum annuum* | 01/01/1973 | *R.pseudosolanacearum* | I | 15 |
| RUN055 | Potato_KE055 | KENYA | *Solanum tuberosum* | 01/01/1960 | *R.solanacearum* | IIA | 7 |
| RUN056 | Potato_KE056 | KENYA | *Solanum tuberosum* | 01/01/1998 | *R.pseudosolanacearum* | III | 20 |
| RUN076 | Tobacco_ZIM076 | ZIMBABWE | *Capsicum annuum* | | *R.pseudosolanacearum* | III | 20 |
| RUN711 | Pelargonium_KE711 | KENYA | *Pelargonium* | 01/01/2004 | *R.solanacearum* | IIB | 1 |
| RUN449 | Pelargonium_KE449 | KENYA | *Pelargonium* | 01/01/2003 | *R.solanacearum* | IIB | 1 |
| RUN100 | Capsicum_KE100 | KENYA | *Capsicum annuum* | 01/01/1974 | *R.pseudosolanacearum* | I | 31 |
| RUN470 | Potato_RW470 | RWANDA | *Solanum tuberosum* | 01/01/1998 | *R.solanacearum* | IIB | 1 |

**Table 2: Summary statistics of eight *Ralstonia solanacearum* species complex strains isolated from symptomatic host plants in sub-Saharan Africa.**

| *Ralstonia solanacearum species complex(RSSC)* bacterium | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CIRAD id | Strain | Contigs | Largest contig | Mean coverage | Total length(bp) | N50 | GC% | Biosample | GenBank Accession(Raw reads) | GenBank Accession (assembly) | Locus_tag prefix |
| **RUN101** | **Capsicum_KE101** | 107 | 318760 | 90.8237 | 5619541 | 126131 | 67.08 | SAMN39646423 | SRR27783136 | JAZKLQ000000000 | V4889 |
| **RUN055** | **Potato_KE055** | 147 | 429721 | 106.036 | 5522821 | 131916 | 66.49 | SAMN39646424 | SRR27783137 | JAZKLP000000000 | V4891 |
| **RUN056** | **Potato_KE056** | 84 | 554125 | 83.2594 | 5562678 | 221609 | 66.74 | SAMN39646425 | SRR27783138 | JAZKLO000000000 | V4890 |
| **RUN076** | **Tobacco_ZIM076** | 112 | 567972 | 118.983 | 5257178 | 131371 | 66.73 | SAMN39646426 | SRR27783139 | JAZKLN000000000 | V4888 |
| **RUN711** | **Pelargonium_KE711** | 112 | 375397 | 104.496 | 5226334 | 131369 | 66.76 | SAMN39646427 | SRR27783140 | JAZKLM000000000 | V4884 |
| **RUN449** | **Pelargonium_KE449** | 114 | 567972 | 106.513 | 5229319 | 117291 | 66.73 | SAMN39646428 | SRR27783133 | JAZKLL000000000 | V4887 |
| **RUN100** | **Capsicum_KE100** | 120 | 375397 | 138.511 | 5243644 | 131372 | 66.74 | SAMN39646429 | SRR27783134 | JAZKLK000000000 | V4885 |
| **RUN470** | **Potato_RW470** | 122 | 263740 | 104.096 | 5558263 | 115013 | 67.04 | SAMN39646430 | SRR27783135 | JAZKLJ000000000 | V4886 |

* Taxon identification was determined by 16S gene matches with the SILVA 16S database (Quast et al., 2013) and calculated using the taxonomic sequence classifier software Kraken (Wood & Salzberg, 2014).

**Acknowledgements**

The sequencing of the bacterial strains was supported by GetGenome and The Sainsbury Laboratory, Norwich, UK, with support from the Gatsby Charitable Foundation and the Biotechnology and Biological Sciences Research Council (BBSRC).

**References**

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, *19*(5), 455–477. https://doi.org/10.1089/cmb.2012.0021

De Boer, S. H. (2006). Book Review: Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex. Edited by Caitilyn Allen, Phillippe Prior, and A.C. Hayward. 2005. 510 pp. APS Press, St. Paul, Minnesota, USA. ISBN 0890543291. US$79.00. *European Journal of Plant Pathology*, *114*(2), 227–228. https://doi.org/10.1007/s10658-005-5039-6

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in Ralstonia solanacearum. In *New Phytologist* (Vol. 187, Issue 4, pp. 920–928). https://doi.org/10.1111/j.1469-8137.2010.03397.x

Kwambai, T. K., Griffin, D., Struik, P. C., Stack, L., Rono, S., Brophy, C., Nyongesa, M., & Gorman, M. (2023). Seed Quality and Variety Preferences Amongst Potato Farmers in North-Western Kenya: Lessons for the Adoption of New Varieties. *Potato Research*. https://doi.org/10.1007/s11540-023-09626-8

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., & Foster, G. D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. In *Molecular Plant Pathology* (Vol. 13, Issue 6, pp. 614–629). https://doi.org/10.1111/j.1364-3703.2012.00804.x

Okeyo, G. O., Charimbu, M. K., Nyaanga, J., & Mendes, T. (2022). Determination of Bioactive Compounds Against Bacterial Wilt of Potato (Ralstonia pseudosolanacearum sp. nov.) in Psidium guajava and Pelargonium zonale Leaf Extracts. *Journal of Agricultural Science*, *14*(11), 64. https://doi.org/10.5539/jas.v14n11p64

Priou, S., Aley, P., Chujoy, E., Lemaga, B., French, E., & French, E. (1999). Integrated control of bacterial wilt of potato. In CIP SlideTraining Series. Lima, Peru. International Potato Center.

Priou, S., Aley, P., Chujoy, E., Lemaga, B., & French, E. R. (n.d.). *INTEGRATED CONTROL OF BACTERIAL WILT OF POTATO*.:https://cipotato.org/wp-content/uploads/2014/10/guiaing.pdf

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1). https://doi.org/10.1093/nar/gks1219

Salanoubat, M., Genin[23], S., Artiguenave, F., Gouzy[3], J., Mangenot, S., Arlat[3], M., Billaultk, A., Brottier, P., Camus[3], J. C., Cattolico, L., Chandler ¶, M., Choisne#, N., Claudel-Renard, C., Cunnac[3], S., Demange, N., Gaspin, C., Lavie[3], M., Moisan, A., Robert, C., … Boucher[3], C. A. (2002). Genome sequence of the plant pathogen Ralstonia solanacearum. In *NATURE* (Vol. 415). www.nature.com

Seemann, T. (2014). Prokka: Rapid prokaryotic genome annotation. *Bioinformatics*, *30*(14), 2068–2069. https://doi.org/10.1093/bioinformatics/btu153

Wood, D. E., & Salzberg, S. L. (2014). *Kraken: ultrafast metagenomic sequence classification using exact alignments*. http://ccb.jhu.edu/software/kraken/.