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The draft genome sequence of *Xanthomonas* species strain Nyagatare, isolated from diseased bean in Rwanda

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One sentence summary: We present the genome sequence of the Nyagatare strain, a bacterial pathogen on beans that may be responsible for a mysterious disease emerging in Rwanda.

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ABSTRACT

We announce the genome sequence for *Xanthomonas* species strain Nyagatare, isolated from beans showing unusual disease symptoms in Rwanda. This strain represents the first sequenced genome belonging to an as-yet undescribed *Xanthomonas* species known as species-level clade 1. It has at least 100 kb of genomic sequence that shows little or no sequence similarity to other xanthomonads, including a unique lipopolysaccharide synthesis gene cluster. At least one genomic region appears to have been acquired from relatives of *Agrobacterium* or *Rhizobium* species. The genome encodes homologues of only three known type-three secretion system effectors: AvrBs2, XopF1 and AvrXv4. Availability of the genome sequence will facilitate development of molecular tools for detection and diagnostics for this newly discovered pathogen of beans and facilitate epidemiological investigations of a potential causal link between this pathogen and the disease outbreak.

Key words: common beans; bacterial canker; *Xanthomonas*; Rwanda

Common bean (*Phaseolus vulgaris*) is an important subsistence and cash crop for smallholder farmers in Rwanda, providing a major source of protein and micronutrients such as iron and zinc (Larochelle and Alwang 2014). In November 2013, farmers in Nyagatare District reported unusual disease on variety ISAR SCB 101 (RWR 2245). Leaf symptoms included curling of upper leaves, wilting, drying and dropping off. There were also brownish and white spots on affected leaves as well as brownish
Figure 1. The genome sequence of *Xanthomonas* sp. Nyagatare. Panel (A) shows a global comparison of the Nyagatare genome sequence against representative previously sequenced *Xanthomonas* genomes. The genome sequences (Pieretti et al., 2009; Song and Yang, 2010; Potnis et al., 2011; Bolot et al., 2013; Darrasse et al., 2013; Vandroemme et al., 2013) were aligned against the Nyagatare genome assembly using BLASTN with an E-value threshold of $1 \times 10^{-6}$. The Nyagatare assembly had first been re-ordered against the *X. axonopodis* pv. *citri* 306 (da Silva et al., 2002) reference sequence using the contig re-ordering function in Mauve (Rissman et al., 2009). The alignments are visualized using BLAST Ring Image Generator (BRIG) (Alikhan et al., 2011). Panel (B) shows the phylogenetic position of the Nyagatare strain based on comparison to previously sequenced *gyrB* genes (Parkinson et al., 2009). Evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood ($-8634.7961$) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 438 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data and ambiguous bases were allowed at any position. There were a total of 524 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). *Xanthomonas* group 1 and 2 as defined by Young and colleagues (Young et al., 2008) are indicated by square brackets as is also species-level clade 1 as defined by Parkinson and colleagues (Parkinson et al., 2009).
to dark necrosis on veins and margins. The stems and branches developed extensive white scabs, which later developed into grey gall-like structures. Green to dark-brown-black streaks and wounds that developed into cankers and necrotic tissues also developed on the stems. The pods developed grey scabs and spots coalescing into large swellings, similar to those on stems. Many of the pods were water soaked, aborted or poorly filled. On dissection, stem vascular tissues were untainted, suggesting that the pathogen is intercellular. A survey by the Rwanda Agriculture Board in November 2013 found that of the 14 sectors of the Nyagatare District were affected. Although the implications were serious for farmers concerned, the overall situation was not yet alarming with no more than 15 ha being affected, but there is concern about possible future spread.

Bacteria were isolated from diseased plant material on YDC (yeast extract dextrose carbonate) medium at CIAT Pathology Laboratory, Uganda. Pathogenicity was demonstrated by inoculation of the isolated strain onto CAL96 beans under glasshouse conditions; symptoms are shown in the Supporting Information. Genomic DNA was sequenced to approximately 58-fold coverage using the Illumina MiSeq with Nextera XT Library Preparation, generating 663,444 pairs of 300-bp reads and assembled into 91 scaffolds with a total length of 4,885,384 bp and an N50 length of 101,745 bp using Velvet 1.2.10 (Zerbino and Birney 2008) followed by gap-filling using GapCloser version 1.12-r6 (Luo et al., 2012). Data are available at GenBank under accession numbers GCA_000764855.1 and JRQI00000001.1.

To investigate the core and variable portions of the genome, we used dnadiff from the Mummer package (Delcher et al., 2002) to perform pairwise sequence comparisons between the Nyagatare strain genome and all previously sequenced Xanthomonas genomes [results are tabulated in Fig. S1 (Supporting Information)]. The highest degree of shared accessory genome was with X. arboricola 3004 (73.73% of genome shared with Nyagatare). Fig. 1A also provides an overview of genomic conservation and variation. The genome with greatest sequence similarity was X. cassavae (Bolot et al., 2013) with 89.16% nucleotide sequence identity. Average nucleotide identity (ANI) values, as calculated by JSpecies (Richter and Rosselló-Móra 2009), between members of a single species usually exceed 95%. The ANI values between Nyagatare and X. cassavae were 87.38% (ANIb) and 89.12% (ANIm). Between Nyagatare and X. arboricola 3004, ANIb was 85.54% and ANIm was 88.84%. Between Nagatare and X. fuscans, the respective values for ANIb and ANIm were 85.82 and 88.66%. Thus, strain Nyagatare does not belong to any of the previously sequenced species and is phylogenetically distinct from previously studied pathogens of common bean (that fall within the species X. axonopodis and X. fuscans). The lack of sequenced genomes with very high sequence similarity to strain Nyagatare precluded high-resolution phylogenomic analysis (Rodriguez-R et al., 2012); however, the availability of an extensive database of sequences for the phylogenetic marker gene gyrB (Parkinson et al., 2009) allowed us to more precisely examine its phylogenetic position. As illustrated in Fig. 1B, the Nyagatare strain falls within Parkinson’s species-level clade 1 (Parkinson et al., 2009), along with little-studied pathogens of Zinnia elegans, Hibiscus esculentus, Cannabis sativa, Helianthus annuus and Nicotiana tabacum (NCPPB strains 2439, 2190, 2877, 1325 and 1068).

Commensurate with its phylogenetic distinctness from previously sequenced Xanthomonas species, the Nyagatare strain has at least 100 kb of genomic sequence that shows little or no sequence similarity to other xanthomonads, as judged by BLASTN searches. This includes a 16.5-kb region located between metB and etfA (JRQI01000003.1 positions 48,238–64,812) harboring genes for lipopolysaccharide (LPS) synthesis that are quite distinct from any previously sequenced LPS synthesis gene cluster (Patil and Sonti 2004). Another example is a 2.3-kb region (JRQI01000032.1 positions 37,278–34,915) that shares 84% nucleotide sequence identity with the large chromosome of Agrobacterium radiobacter K54 (GenBank: CP000628.1), and similar levels of identity with several Rhizobium species, but shares no detectable sequence similarity with any available Xanthomonas sequences in the NCBI databases.

Virulence factors described in previously sequenced Xanthomonas genomes include effector proteins that are substrates of the type-III secretion system (T3SS) (White et al., 2009). The Nyagatare genome encodes an apparently complete T3SS (Fig. S2, Supporting Information). Based on TBLASTN searches between the genome of the Nyagatare strain and Ralph Koebnik’s catalogue of known T3SS effectors (http://www.xanthomonas.org/t3e.html), there are homologues of only three: AvrBs2 (73% identity between GenBank: CAJ21683.1 and JRQI01000008.1: 30,926 to 33,058), XopF1 (66% identity between CAJ22045.1 and NC003340) and an open reading frame (JRQI01000001.1 positions 38,866 to 39,942) encoding a protein with 87% amino-acid sequence identity to AvrXv4 which has only previously been reported in genomes of X. eucalyctatoria (Astua-Monge et al., 2000) and X. perforans (Potnis et al., 2011).

In conclusion, we present a draft-quality genome sequence for the Nyagatare strain. This is the first genome sequence representing Parkinson’s species-level clade 1, and as such its availability will aid the study of this as-yet undescribed candidate new species. Furthermore, this strain may be responsible for the mysterious disease emerging as a potentially serious threat to beans, an important subsistence crop. Availability of the genome sequence will facilitate development of molecular tools for detection and diagnostics thus enabling researchers to test for an epidemiological link between this strain and the disease.

SUPPLEMENTARY DATA
Supplementary data is available at FEMSLE online.

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Conflict of interest statement. None declared.

REFERENCES
Astua-Monge G, Minsavage G V, Stall RE, et al. Resistance of tomato and pepper to T3 strains of Xanthomonas campestris


Song C, Yang B. Mutagenesis of 18 type III effectors reveals virulence function of XopZ(PXO99) in Xanthomonas oryzae pv. oryzae. Mol Plant Microbe In 2010;23:893–902.