Complete Genome Sequence Data of *Xenorhabdus budapestensis* Strain C72, a Candidate Biological Control Agent from China

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Abstract

Xenorhabdus budapestensis strain C72 isolated from the entomopathogenic nematode of *Steinernema bicornutum* possesses an excellent biocontrol effect on southern corn leaf blight. However, its genomic information is lacking. Here, we report a high-quality complete and annotated genome sequence of *X. budapestensis* strain C72. Fifteen secondary metabolite biosynthetic gene clusters are identified in the genome, which are responsible for the production of a diverse group of antimicrobial compounds to help host plants against agricultural pathogenic diseases. This genome sequence could contribute to investigations of the molecular basis underlying the biocontrol activity of this *Xenorhabdus* strain.

Resource Announcement

The bacteria of genus *Xenorhabdus* belong to the family *Enterobacteriaceae* and establish obligate mutualistic associations with entomopathogenic nematodes of the genus *Steinernema*. They are well known for their potential to produce a variety of bioactive compounds with antimicrobial and insecticidal activities (Dreyer et al. 2018; Liu et al. 2000). In previous decades of research, the symbionts were commercially and successfully used as biological control agents in agriculture to kill a wide variety of pest insect species in China, North America, Europe, Australia, and Africa (Bera et al. 2014; Garriga et al. 2020). The symbiotic *Xenorhabdus* bacteria not only contribute to the nematode's ability to kill the host by releasing insecticidal proteins, immunosuppressors, and lytic enzymes but also restrain the growth of other microorganisms in the absence of nematodes by the production of antimicrobial compounds (Shi and Bode 2018).

Various kinds of biological molecules have been isolated and described from the genus *Xenorhabdus*. The main antimicrobial compounds include ribosomal-encoded benzylidenacetone (Ji et al. 2004), xenocin, and bicornutin (Böszörményi et al. 2009; Rathore 2013) and non-ribosomally produced xenematides (Xi et al. 2019), fabclavines (Fuchs et al. 2014), nematophin (Li et al. 1997), xenocoumacin (Park et al. 2009), peptide-antimicrobial-*Xenorhabdus* lipopeptides (Fuchs et al. 2011), and rhabdopeptides (Zhao et al. 2018). Particular attention should be paid to some members of the genus *Xenorhabdus* such as *Xenorhabdus budapestensis* and *X. nematophila* for their antiphytopathogenic properties (Ji et al. 2004; Vozik et al. 2015); these species need to be comprehensively investigated for the possibility of using them as alternatives to chemical pesticides in agriculture.

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Keywords

biocontrol, genome, southern corn leaf blight, *Xenorhabdus budapestensis*

Table 1. Identified secondary metabolite regions in genome of *Xenorhabdus budapestensis* C72 using antiSMASH 5.2.0

Gene cluster	Most similar known cluster ^a	Similarity (%)	Cluster types ^b	Gene sequence locations	
Region 1	Luminide	100	NRPS	88,888	150,983
Region 2	Safracin A/safracin B	20	NRPS-like	414,262	454,892
Region 3	Taxllaid A	4	Lasso peptide	696,101	718,483
Region 4	Xenocoumacin 1/ xenocoumacin II	14	NRPS, T1PKS	1,455,717	1,532,527
Region 5	Fabclavine Ia	100	NRPS, T1PKS	1,571,752	1,691,805
Region 6	Yersiniabactin	5	NRPS, T1PKS	2,003,086	2,064,878
Region 7	Glidopeptin	25	NRPS	2,164,883	2,253,962
Region 8	Pyrrolizixenamide A	100	NRPS	2,262,133	2,307,370
Region 9	Nematophin	37	NRPS	2,358,628	2,412,306
Region 10	Neneprotides A-C	55	NRPS	2,472,098	2,585,011
Region 11	ND		NRPS, AT-PKS	2,630,477	2,706,071
Region 12	Xenoamicin A/ xenoamicin B	25	NRPS	2,716,887	2,768,694
Region 13	ND		Thiopeptide	2,866,887	2,892,869
Region 14	Putrebactin/ avaroferrin	100	Siderophore	3,180,981	3,205,638
Region 15	ND		β -Lactone	3,508,153	3,533,766

^a ND = not determined.

^b NRPS = nonribosomal peptide synthetase and T1PKS = type I polyketide synthase.

We previously isolated and identified a strain *X. budapestensis* C72 from *Steinernema bicornutum* in Changbai Mountain, Jilin, China, which was considered a novel source of highly efficient antimicrobial compounds against plant pathogens. In our previous study, this strain possessed broad-spectrum antifungal activities in vitro and exhibited excellent biocontrol efficacy against *Bipolaris maydis*, which is the causal agent of southern corn leaf blight in the field (Li et al. 2021). It is also the first report of *Xenorhabdus* inhibiting *B. maydis* (Li et al. 2021). The antagonistic property of *X. budapestensis* C72 is attributed to the production of bioactive metabolites (lytic enzymes and antibiotics) and, hence, the isolate was molecularly and phylogenetically characterized (16S ribosomal RNA [rRNA] GenBank accession number MT967912.1) (Li et al. 2021). To date, only one draft genome sequence of *X. budapestensis* DSM 16342 strain has been reported (Tobias et al. 2017). Because very little is currently known about the beneficial properties of the *X. budapestensis* sp., we sequenced the whole genome of the isolate *X. budapestensis* C72 to further explore its genetic traits and biosynthetic gene clusters related to biologically active substances.

The *X. budapestensis* C72 strain was obtained by single-colony isolation on nutrient agar supplemented with bromothymol blue and triphenyl-2,3,5-tetrazolium chloride plates and was aerobically cultured in Luria-Bertani broth at 28°C for 2 days. Genomic DNA was extracted using a EasyPure Bacteria Genomic DNA Kit (TransGEN, Beijing, China), following the manufacturer's protocols, with RNase A treatment. The quality and quantity of the total DNA was evaluated via agarose gel electrophoresis and a Quant-iT PicoGreen dsDNA assay kit, respectively (Invitrogen, Carlsbad, CA, U.S.A.). Genomic DNA was sequenced and analyzed by OE Biotech Co., Ltd. (Shanghai, China). The sequencing libraries were prepared with the SMRTbell template prep kit 1.0 as directed by the manufacturer (Pacific Biosciences, Menlo Park, CA, U.S.A.), and single-molecule real-time (SMRT) sequencing was conducted on the PacBio Sequel platform. Default software settings were used throughout unless otherwise noted.

The high-quality filtered reads were assembled to generate one contig without gaps by using SMRT Analysis 2.3.0 (Pacific Bioscience), which yielded a total of 508,934 subreads (N_{50} value = 4,366,413) with an average length of 4,067 bp (total = 2,344,639,223 bp), providing over 500-fold genome coverage. The size of the *X. budapestensis* C72 genome was 4,366,413 bp with a G+C content of 43.14%. The genomic sequence was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (v5.1) based on the Best-placed reference protein set and GeneMarkS+ (Tatusova et al. 2016). In all, 3,596 protein coding genes were predicted. Then, the transfer RNA (tRNA), rRNA, and small nuclear RNA (sRNA) were detected by tRNAscan-SE (v1.3.1) (Lowe and Eddy 1997), RNAmmer (v1.2) (Lagesen et al. 2007), and Rfam (v10.0) (Griffiths-Jones 2003), respectively. Among the total 104 noncoding RNA genes,

22 genes encoded for rRNAs, 78 encoded for tRNAs, and 4 were identified as sRNAs. In total, 11 prophage sequences were detected using PhiSpy (v2.3) (Akhter et al. 2012). Analysis of secondary metabolite biosynthetic gene clusters was done with antiSMASH (v5.2.0) (Blin et al. 2019). The biosynthetic clusters (15 clusters) and bioproducts (e.g., fabclavine Ia, pyrrolizixenamide A, and putrebactin) present in *X. budapestensis* C72 were predicted and are shown in Table 1. The different types of secondary metabolite clusters such as nonribosomal peptide synthetase (NRPS), polyketide synthase (PKS), and siderophore presented in the C72 strain genome sequence may play a crucial role in antimicrobial activity.

The C72 strain genome is the first *X. budapestensis* complete genome reported from China. The C72 genome information will provide a valuable resource for future studies on the understanding of the molecular basis of both the application of biocontrol and symbiotic relationship between *X. budapestensis* and their host nematodes.

Data availability. The complete genome sequence for C72 has been deposited into GenBank under the accession number CP072455 (genome annotation is available at <https://www.ncbi.nlm.nih.gov/nuccore/2021543890/>). The BioProject and BioSample designations for this project are PRJNA625211 and SAMN14595202, respectively. Raw reads have been deposited into NCBI Sequencing Read Archive (SRA accession number SRR13921535) in association with BioProject PRJNA625211.

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