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Genome Note

Draft genome sequence of *Dichelobacter nodosus* JKS-07 serogroup E from India

Shakil A. Wani[a], Zahid Kashoo[a], Shaheen Farooq[a], Sabia Qureshi[a,*], Basharat Bhat[b,*], M.Isfaqul Hussain[a], Aasim Habib[a], Shafkat Majeed Khan[a], Javeed A. Malla[a], Md Zubbair Malik[c], Bilal Ahmad Dar[a]

[a] *Anaerobic Laboratory, Division of Veterinary Microbiology & Immunology, FVSc & AH Shuhama (Alusteng), Sher-e-Kashmir University of Agricultural Sciences and Technology–Kashmir (SKUAST-K), J&K 190006, India*
[b] *Division of Animal Genetics and Breeding, FVSc & AH Shuhama (Alusteng), SKUAST-K, J&K 190006, India*
[c] *School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Delhi 110067, India*

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ABSTRACT

Objectives: Dichelobacter nodosus is an anaerobic bacterium with fastidious growth requirements that is the principal cause of footrot associated with lameness in sheep and goats. In India, *D. nodosus* serogroups B and E have been recorded as major causes of footrot. Here we report the draft genome sequence of a *D. nodosus* serogroup E strain (JKS-07) from a case of virulent footrot in India.
Methods: The whole genome of the *D. nodosus* JKS-07 serogroup E was sequenced using an Illumina HiSeq 2500 platform and was annotated according to functional gene categories. De novo genome assembly and annotation were performed using Perl scripts developed in-house using the Nr/Nt and UniProt databases.
Results: The assembled genome is 1 389 350 bp and contains 1301 genes. The genome has 45 tRNAs and 9 rRNAs. The draft genome sequence will provide insight into the various genes and regulators involved in *D. nodosus* growth and survival.
Conclusion: Information on the genome of the *D. nodosus* serogroup E strain is important bearing in mind the fact that both serogroups B and E are associated with virulent footrot, either alone or frequently together. In order to develop an efficacious vaccine against virulent footrot, it is essential to know the serological diversity as well as the virulence status of the *D. nodosus* strains. Serogroups B and E are potential vaccine candidates to mitigate ovine footrot in India.

The Gram-negative anaerobic bacterium *Dichelobacter nodosus* is the causative agent of ovine footrot, a disease of major economic importance to the sheep industry globally [1]. The disease is considered to be chronic and endemic causing substantial economic losses through loss of body weight, condition and wool growth, decreased lambing percentage, and reduced value at sale of the affected sheep [2].

Dichelobacter nodosus is a fastidious anaerobic bacterium requiring special media and conditions for growth. Currently, *D. nodosus* strains are classified into at least 10 serogroups (A–I and M) based on the fimbrial antigen. These serogroups are further divided into 21 serotypes (A1, A2, B1, B2, B3, B4, B5, B6, C1, C2, D, E1, E2, F1, F2, G1, G2, H1, H2, I and M) [3]. The *D. nodosus* fimbrial antigen is highly immunogenic and is the major host-protective immunogen [4].

The virulence factors of *D. nodosus* include extracellular subtilisin-like serine proteases (or subtilases), type IV fimbriae, and the vrl and vap genomic islands [5]. Virulent strains of *D. nodosus* produce three homologous extracellular subtilases, namely AprV5, AprV2 and BprV. The AprV2 protease is thermostable and is responsible for elastase activity [6]. AprV2 differs from its benign counterpart AprB2 by a single amino acid substitution (Tyr92Arg). At the gene level, the difference between the protease gene variants *aprV2* (accession no. L38395) and *aprB2* consists of a 2-bp change from TA to CG at position 661–662 [7]. The other two proteases are acidic protease 5 (AprV5 and AprB5) and basic protease (BprV and BprB) from virulent and benign strains, respectively.

Based on the production of thermostable proteases and/or the presence of the virulence-specific integrase A (*intA*) gene, *D. nodosus* strains can be categorised as virulent, intermediate or

* Corresponding authors.
E-mail addresses: qureshi.sabia@gmail.com (S. Qureshi), bb284@snu.edu.in (B. Bhat).

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benign, which are responsible for the corresponding forms of the disease [8].

Whole-genome information pertaining to circulating strains is important to understand the relationship between virulent and benign strains as well as the relationship of isolates from different geographical regions.

Currently used treatment for footrot as recommended by Farm and Animal Health is mainly based on footbathing with 10% (w/v) zinc sulphate and 10% (w/v) copper sulphate. Tetracycline and enrofloxacin antibiotics are used in severe cases for systemic treatment of footrot [9]. However, *Fusobacterium* spp. and *Dichelobacter* spp., the primary pathogens incriminated in the aetiology of footrot, are not completely susceptible to nitroimidazoles. There are reports of metronidazole resistance encoded by the *nim* gene, found both on the chromosome and on plasmids, with both the markers being transferable [10]. This is important in view of metronidazole being widely used in veterinary medicine for its activity against anaerobic bacteria [11].

The strain from India sequenced in this study belongs to serogroup E. The overall prevalence of serogroup E in ovine footrot ranges from 15–20% and this serogroup is often isolated in combination with serogroup B, particularly from footrot lesions with a lesion score of 4 [3]. Genomic DNA of the *D. nodosus* serogroup E strain (JKS-07B) was extracted using a Wizard® Genomic DNA Purification Kit (Promega Corp., Madison WI). Whole-genome sequencing of the strain was carried out with 2 × 100-bp paired-end multiplex sequencing on an Illumina HiSeq 2500 platform (Illumina Inc., San Diego, CA). Genome annotation was performed using Perl scripts developed in-house against the Nr/Nt and UniProt databases. Raw reads were transformed to clean reads using Perl scripts developed in-house by removing the adapters sequences, low-quality reads and those containing undetermined bases. All clean reads (Phred quality scores >30 and length >25 bp) were mapped to the *D. nodosus* reference genome assembly VCS1703A (GenBank accession no. NC_009446.1) downloaded from the National Center for Biotechnology Information (NCBI) using Bowtie-v2.2.6 29 [12].

A total of 96% reads were mapped successfully to the reference genome. The assembled genome is 1 389 350 bp in length with an average G + C content of 44.4% and a total of 1301 genes. The genome has 45 tRNAs [13] and 9 complete rRNAs (Table 1). This draft genome sequence was compared with the *D. nodosus* genome sequence VCS1703A [14] with a total length of 1 389 350 bp encoding 1299 protein-coding genes. The data set submitted to NCBI includes the assembled consensus sequence of *D. nodosus* JKS-07B in fasta format. The genome sequence can be accessed at NCBI using the accession no. **SRX3594754**. The draft genome and annotations are available on the ribosomal multilocus sequence typing (rMLST) genome database (ID no. 116; http://pubmlst.org/rmlst/). A comparative genome analysis of *D. nodosus* ovine serogroup E isolated from Kashmir, India, showed high sequence conservation (95% sequence similarity, E-value = 0.0) with the reference genome.

Table 1
Genome characteristics of *Dichelobacter nodosus* JKS-07B serogroup E, and resources used.

Name	Genome characteristic
NCBI BioProject ID	SAMN08389740
SRA ID	SRR6506170
Sequencing platform	Illumina HiSeq
Total no. of reads	7 328 461 PE
Total cleaned reads	6 960 106 PE
Cleaned read length	20–100 bp
Genome coverage	>100×
Mapped reads	6 682 111 PE
Estimated genome size	1.406 Mb
GC content	44.4%
Protein-coding genes	1243
tRNA-coding genes	45
Complete rRNA	9
ncRNA	4
Pseudogenes	21

PE, paired-end; ncRNA, non-coding RNA.

This draft genome was compared with the genome sequence available for *D nodosus* strain ATCC 25549, strain VPI 2340 [11342] [15] with a total length of 1.38935 Mb encoding 1348 non-redundant genes. There are 1227 identical genes in both strains (ca. 60% sequence similarity, E-value <0.0005). However, 16 genes identified by blastX in JKS-07 serogroup E (**SRX3594754**), comprising mainly lactate dehydrogenase (protein ID AXM45131.1), type I-F CRISPR-associated helicase Cas3 (protein ID AXM45024.1), FeoB-associated Cys-rich membrane protein (protein ID AXM45066.1), transposase, tRNA pseudouridine synthase TruA (protein ID AXM45575.1), 50S ribosomal protein L36 (protein ID AXM45863), translational initiation factor IF-3 (protein ID AXM45407.1), tRNA pseudouridine synthetase L36 (protein ID AXM45863.1) and other hypothetical proteins were not found in ATCC 25549, strain VPI 2340 [11342].

Cas3 been found as a key element in the defence of bacteria and helps to maintain its integrity. The gene coding for lactate dehydrogenase (AXM45131.1) has a role in energy generation under extreme conditions. Two genes encoding for the paraquat-inducible protein A (AXM45380.1/AXM46132.1) also help to maintain membrane integrity. Translation initiation factor IF-3 (AXM45407.1) also found among the 16 novel genes may help in the translation of genes required for survival under critical conditions. Bacterial helicases play an important role in bacterial cell survival during antibiotic damage thus contributing to virulence. FeoB and FeoA proteins play a role in ferrous iron transport and contribute to bacterial virulence, with ferrous iron (Fe^{2+}) being more abundant under anaerobic conditions or at low pH for organisms that must combat oxygen limitation for their everyday survival. Transposases are mobile genetic elements suggested to have an important role in bacterial genome plasticity and host adaptation. All 16 of these novel proteins may contribute towards the increased virulence of JKS-07B serogroup E (**SRX3594754**).

The first *D. nodosus* genome to be sequenced was strain VCS1703A (NC_009446.1). Although it is not the type strain, it has been used in several virulence studies. The genome of *D. nodosus* is very small (ca. 1 400 000 bp) and one-fifth of the genome is believed to probably have been acquired by lateral gene transfer of an incorporated Mu-like bacteriophage. Genes for amino acid transportation have, however, been identified and it is proposed that the extracellular proteases provide the cell with amino acids by degradation of host proteins [14].

At present (September 2017) the *D. nodosus* PubMLST database contains 171 isolates with 115 sequence types. The database suggests a high level of diversity with a low level of recombination, which is reflected in the grouping of isolates and branch lengths shown in the core genome MLST (cgMLST) and whole genome MLST (wgMLST) analyses [16].

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Competing interests

None declared.

Ethical approval

Not required.

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