



SHORT GENOME REPORT

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Non-contiguous finished genome sequence of *Ornithobacterium rhinotracheale* strain H06-030791

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Abstract

The Gram-negative, pleomorphic, rod-shaped bacterium *Ornithobacterium rhinotracheale* is a cause of pneumonia and airsacculitis in poultry. It is a member of the family *Flavobacteriaceae* of the phylum "Bacteroidetes". *O. rhinotracheale* strain H06-030791 was isolated from the lung of a turkey in North Carolina in 2006. Its genome consists of a circular chromosome of 2,319,034 bp in length with a total of 2243 protein-coding genes and nine RNA genes. Genome sequences are available for two additional strains of *O. rhinotracheale*, isolated in 1988 and 1995, the latter described in a companion genome report in this issue of SIGS. The genome sequence of *O. rhinotracheale* strain H06-030791, a more contemporary isolate, will be of value in establishing core and pan-genomes for *O. rhinotracheale* and elucidating its evolutionary history.

Keywords: *Ornithobacterium rhinotracheale*, Respiratory disease, Poultry, Genome sequence

Introduction

Ornithobacterium rhinotracheale has been implicated as a cause of respiratory disease in domesticated fowl since at least 1981 [1]. Initially characterized as a phenotypically unusual bacterium of uncertain identity [2], Vandamme et al. [3] further characterized and named *O. rhinotracheale* in 1994. *O. rhinotracheale* is a global pathogen in farmed turkeys and chickens as well as a variety of other domesticated and wild birds, including chukar partridges, geese, ducks, guinea fowl, ostriches, gulls, pheasants, partridges, pigeons, quail, rooks, and falcons [4,5]. Based on the reactivity of heat-extracted antigens with monospecific antisera, 18 serotypes of *O. rhinotracheale* have been defined, designated as A through R [1,4], although not all isolates are typeable. The most common clinical signs of disease related to *O. rhinotracheale* are tracheitis, pneumonia, airsacculitis, sinusitis, and pericarditis [1,4]. The bacterium is responsible for substantial economic losses to the poultry industry worldwide, resulting from decreased egg production, reduced eggshell quality and hatchability, reduced weight gain, increased mortality, and increased condemnation rates [6-9]. Whole-cell bacterin and live, attenuated vaccines

have met with variable success, likely due to the lack of cross-protection against heterologous serotypes. Recent studies have identified antigens that appear to provide cross-protective immunity when formulated as a recombinant, multi-component subunit vaccine [10].

O. rhinotracheale strain H06-030791 was isolated in 2006 from the lung of a turkey in North Carolina and subsequently determined to be serotype A in the laboratory of Dr. K. V. Nagaraja at the University of Minnesota, St. Paul, MN. Further study revealed that growth of *O. rhinotracheale* strain H06-030791 *in vitro* is unaffected by the presence of an iron chelator [11] a phenotype not shared by most of the other field isolates tested. Whether or how this attribute plays a role in disease is not yet clear. Although *O. rhinotracheale* has generally been considered nonhemolytic on blood agar, Tabatabai et al. [12] documented strong β -hemolytic activity of *O. rhinotracheale* strain H06-030791 and suggested that a hemolysin-like protein may function as a virulence factor. Here we present a description of the non-contiguous finished genome of *O. rhinotracheale* strain H06-030791 and its annotation. This isolate (alias P5932) was provided to the National Animal Disease Center by the University of Minnesota and is available from the National Animal Disease Center Biological Agent Archive.

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Organism information

Classification and features

The genus *Ornithobacterium* belongs to the class *Flavobacteriia* and is in the family *Flavobacteriaceae* [13] (Table 1). *O. rhinotracheale* is the sole species within the genus. Phylogenetic analysis based on 16S ribosomal RNA of *O. rhinotracheale* and other genera within the *Flavobacteriaceae* family is shown in

Figure 1. The 16S rRNA sequences of *O. rhinotracheale* strain H06-030791 and the type strain, LMG 9086, share 99.9% nucleotide sequence identity. Three rRNA loci were found in the genome of *O. rhinotracheale* strain H06-030791. All *O. rhinotracheale* strains in Figure 1 were isolated from turkeys, with the exception of strain LMG 11554, which was cultured from a rook.

Table 1 Classification and general features of *O. rhinotracheale* strain H06-030791 in accordance with the MIGS recommendations [14]

MIGS ID	Property	Term	Evidence code ^a
	Current classification	Domain " <i>Bacteria</i> "	TAS [15,16]
		Phylum " <i>Bacteroidetes</i> "	TAS [17,18]
		Class " <i>Flavobacteriia</i> "	TAS [19,20]
		Order <i>Flavobacteriales</i>	TAS [21,22]
		Family <i>Flavobacteriaceae</i>	TAS [23-25]
		Genus <i>Ornithobacterium</i>	TAS [26,27]
		Species <i>rhinotracheale</i>	TAS [26,27]
MIGS-7	Subspecific genetic lineage (strain)	Strain H06-030791	TAS [11]
		Serotype A	IDA
	Gram stain	Negative	TAS [1,4]
	Cell shape	Pleomorphic rod	TAS [1,4]
	Motility	Nonmotile	TAS [1,4]
	Sporulation	Non-sporulating	TAS [1,4]
	Temperature range	Mesophile (30°C-42°C)	TAS [1,4]
	Optimum temperature	37°C	TAS [1,4]
MIGS-6.2	pH range; Optimum	7.2-7.6 (BHI); 7.4	TAS [1], IDA
	Carbon source	Saccharolytic (glucose)	TAS [4]
MIGS-6	Habitat	Respiratory tract of birds worldwide	TAS [1,4]
MIGS-6.3	Salinity	Growth in BHI broth, (0.75% salts)	TAS [1], IDA
MIGS-22	Oxygen requirement	Microaerophilic, anaerobic, or aerobic	TAS [1,4]
	Energy metabolism	Chemoorganotroph	TAS [4]
MIGS-15	Biotic relationship	Parasitic	TAS [4]
MIGS-14	Pathogenicity	Pneumonia, airsacculitis, tracheitis, pericarditis	TAS [1,4]
MIGS-16	Specific host	Poultry	TAS [1,4]
MIGS-18	Health status of host	Symptomatic	TAS [11]
	Biosafety level	2 t	TAS [28]
MIGS-19	Trophic level	Chemoheterotroph	TAS [4]
MIGS-23.1	Isolation	Turkey lung	TAS [11]
MIGS-4	Geographic location	North Carolina, USA	TAS [11]
MIGS-5	Time of sample collection	2006	NAS
MIGS-4.1	Latitude	Not reported	
MIGS-4.2	Longitude	Not reported	
MIGS-4.3	Depth	Not reported	
MIGS-4.4	Altitude	Not reported	

^aEvidence codes - IDA: Inferred from Direct Assay by Dr. K. V. Nagaraja, University of Minnesota, St. Paul, MN; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). Evidence codes are from the Gene Ontology project [29].

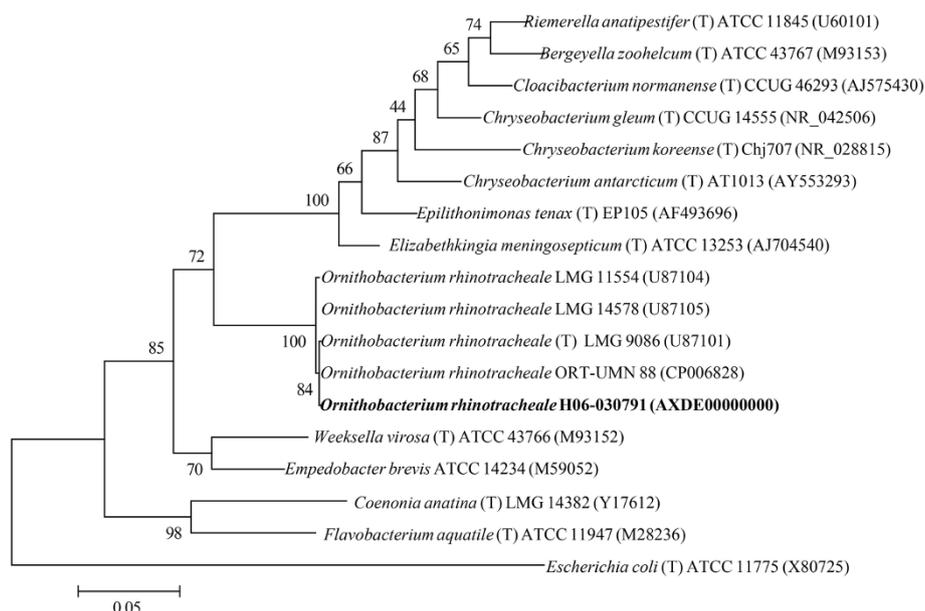


Figure 1 Phylogenetic tree based on 16S rRNA showing the position of *O. rhinotracheale* strain H06-030791 (highlighted in bold) in relation to other *O. rhinotracheale* isolates for which sequence is available and to the type strains (T) of closely related species and genera within the family Flavobacteriaceae. *Escherichia coli* (a member of the Enterobacteriaceae family) was included as an outgroup. An internal region of the 16S RNA gene (1251 bp with no gap-containing sites) was aligned using CLUSTALW and phylogenetic inferences were obtained using the maximum likelihood method and the Jukes-Cantor model within MEGA version 5.10 software [37]. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. GenBank accession numbers for the DNA sequences used are shown in parentheses. The scale bar represents 5% substitution per nucleotide position.

O. rhinotracheale strain H06-030791 is a Gram-negative, pleomorphic rod, when grown in broth medium, ranging from 1.57-2.19 μm (mean, 1.93 μm) in length and 0.42-0.64 μm (mean, 0.48 μm) in width (Figure 2). The bacterium is nonmotile and microaerophilic, and prefers a 7.5% CO_2 humidified atmosphere from 30°C to 42°C for growth. Colonies are approximately 1 mm in diameter and yellowish in color after 48 h incubation at 37°C on blood agar. Although *O. rhinotracheale* type strain LMG 9086 is nonhemolytic [3], *O. rhinotracheale* strain H06-030791 is β -hemolytic on 5% sheep blood agar [12].

Biochemical tests for *O. rhinotracheale* strains can yield variable results [1]. After seven days of incubation at 37°C, *O. rhinotracheale* strain H06-030791 is weakly acidic on a triple sugar iron agar slant and does not produce hydrogen sulfide or gas. Dextrose is weakly fermented with or without the addition of 2% chicken serum, while galactose and lactose are weakly fermented only with the addition of 2% chicken serum. Sucrose, sorbitol, xylose, and mannitol are not fermented with or without the addition of 2% chicken serum. The isolate is lysine decarboxylase positive, ornithine decarboxylase negative, and urease negative.

Genome sequencing and annotation

Genome project history

Genome sequences are currently available for only two additional strains of *O. rhinotracheale*, the type strain LMG 9086 (isolated in 1988) and strain ORT-UMN 88

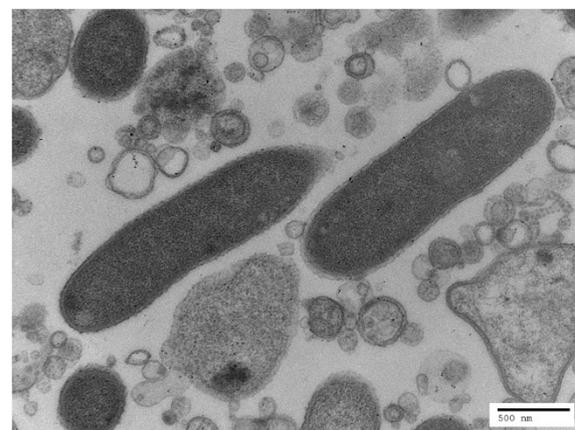


Figure 2 Transmission electron micrograph of *O. rhinotracheale* strain H06-030791 cells cultured in broth, using a Tecnai G² (FEI, Hillsboro, OR) at an operating voltage of 80 kV. The average length of representative cells was 1.93 μm and the average width was 0.48 μm . The scale bar represents 500 nm.

(isolated in 1995; see companion report in this issue of SIGS). *O. rhinotracheale* strain H06-030791 was selected for sequencing to provide a basis for comparative analysis of contemporary versus historical isolates. Additionally, *O. rhinotracheale* strain H06-030791 possesses phenotypic traits unique from those of *O. rhinotracheale* strain LMG 9086 and *O. rhinotracheale* strain ORT-UMN 88 [11,12] that may permit a more accurate representation of the core and pan-genomes of *O. rhinotracheale*. The Whole Genome Shotgun project and non-contiguous finished genome sequence of *O. rhinotracheale* strain H06-030791 has been deposited in DDBJ/EMBL/GenBank under accession no. AXDE00000000. Sequencing, finishing, and final annotation were performed at the DNA Facility of Iowa State University and the National Animal Disease Center, Ames IA. A summary of the project information is given in Table 2.

Growth conditions and DNA isolation

A clonal population of *O. rhinotracheale* strain H06-030791 was derived from a single colony serially passaged three times and archived at -80°C for future analysis. The bacterium was grown on 5% sheep blood agar plates (Becton, Dickinson and Company, Sparks, MD) incubated for 48 h at 37°C with 7.5% CO_2 and 15% humidity. Colonies were used to inoculate 5 ml of brain heart infusion broth in a snap-cap tube which was incubated at 37°C for 24 h with rotation at 100 rpm. Twenty ml of these BHI cultures were inoculated into 100 ml of fresh BHI in a 250-ml flask and incubated at 37°C for 48 h with rotation at 75 rpm (final $\text{OD}_{600} = 0.278$). An aliquot was plated on 5% sheep blood agar to confirm purity and 20 ml was removed for DNA preparation. Cells were pelleted successively into one 2-ml centrifuge tube at $16,000 \times g$. Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega

Table 3 Genome statistics of *O. rhinotracheale* strain H06-030791

Attribute	Genome (total)	
	Value	% of total ^a
Genome size (bp)	2,319,034	100.00%
DNA coding (bp)	2,100,363	90.57%
DNA G + C (bp)	800,726	34.53%
Total genes	2300	100.00%
Protein-coding genes	2243	97.52%
RNA genes	9	0.39%
rRNA operons	3	
tRNA genes	42	1.83%
Pseudo genes	6	0.27%
Genes with function prediction	1058	47.17%
Genes assigned to COGs	1384	61.70%
Genes assigned Pfam domains	1487	66.30%
Genes with signal peptides	254	11.32%
Genes with transmembrane helices	471	21.00%
CRISPR repeats	1	

^aThe total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

Corporation, Madison, WI) with the following modifications: the cell pellet was resuspended in 480 μl of 200 mM EDTA, 60 μl of 10 mg/ml lysozyme, and 60 μl of double distilled water prior to lysis, then 10 μl of 10 mg/ml RNase solution was added to the cell lysate. The precipitated genomic DNA was rehydrated at 65°C for 1 h in 10 mM Tris-HCl, pH 8.5, evaluated on a 6% agarose gel to verify the lack of low molecular weight fragments, and quantified using the Quant-iT PicoGreen ds DNA Assay Kit (Invitrogen, Carlsbad, CA).

Table 2 Project information of *O. rhinotracheale* strain H06-030791

MIGS ID	Property	Term
MIGS-31	Finishing quality	Non-contiguous finished
MIGS-28	Libraries used	Three genomic libraries: two shotgun libraries, one mate-pair library (8 kb insert size)
MIGS-29	Sequencing platforms	Illumina GA II, Roche GS FLX Titanium, Sanger
MIGS-31.2	Fold coverage	48x (26x Roche FLX, 23x Illumina); final SEQuel error correction with 100x Illumina
MIGS-30	Assemblers	MIRA v3.4.0, Roche gsAssembler v2.8
MIGS-32	Gene calling method	GeneMarkS + (NCBI PGAP)
	GenBank ID	AXDE00000000
	GenBank Date of Release	September 22, 2014
	GOLD ID	Gi0071045
	NCBI project ID	219465
	Project relevance	Poultry respiratory pathogen
MIGS-13	Source material identifier	H06-030791

Genome sequencing and assembly

A scaffolded genome was assembled using MIRA v. 3.4 [30] and the Roche gsAssembler v. 2.6 to achieve 49 × total genome coverage through the assembly of Roche GS FLX shotgun, GS FLX large insert (8.3 kb) mate pair, Illumina 75-bp single direction, and Illumina 2 × 75 bp paired-end sequencing reads. Some of remaining sequencing gaps in the scaffolded assembly were PCR amplified and sequenced by the Sanger method. GAP5 [31], from the Staden Package, was used as the editor for incorporating the gap-closing sequences, ultimately resulting in a high quality assembly consisting of eight contigs and seven gaps. (The genome start and end points are in a complete contig that was intentionally split to facilitate comparisons to a completed genome of the same genus and species.) Base calling errors in the genome assembly were corrected by using SEQuel [32] to map Illumina reads back to the contigs at approximately 100 × total coverage.

Genome annotation

The assembled genome was submitted to the National Center for Biotechnology Information (Bethesda, MD) through the Whole Genome Shotgun genome sequencing portal [33] and annotated with the NCBI Prokaryotic Genome Annotation Pipeline. Signal peptides were distinguished from transmembrane regions by using SignalP 4.0 software [34], transmembrane helices were predicted with the method of Krogh et al. [35], and the CRISPR motif was discovered with a web tool described by Griss et al. [36].

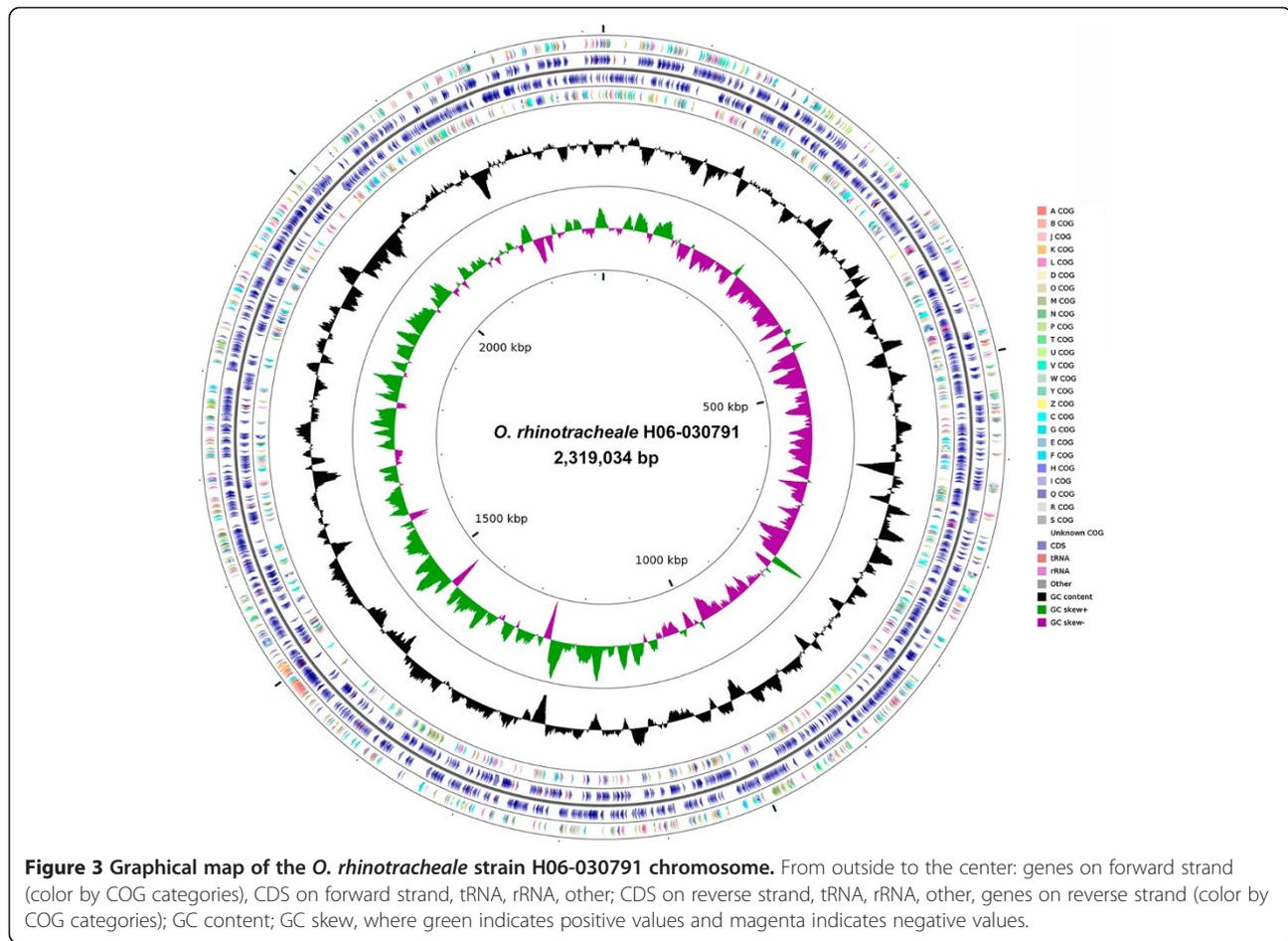
Genome properties

The genome properties and statistics of *O. rhinotracheale* strain H06-030791 (Accession AXDE00000000) are presented in Tables 3 and 4 and Figure 3. The non-contiguous finished genome consists of a circular 2,319,034 bp chromosome with a 34.53% G + C content and no plasmids. Of the 2,300 genes predicted, 2,243

Table 4 Number of genes associated with the 25 general COG functional categories of *O. rhinotracheale* strain H06-030791

Code	Value	% age ^a	Description
J	133	5.7	Translation, ribosomal structure and biogenesis
A	0	0	RNA processing and modification
K	47	2.01	Transcription
L	118	5.06	Replication, recombination and repair
B	0	0	Chromatin structure and dynamics
D	19	0.81	Cell cycle control, cell division, chromosome partitioning
Y	0	0	Nuclear structure
V	36	1.54	Defense mechanisms
T	24	1.03	Signal transduction mechanisms
M	122	5.23	Cell wall/membrane biogenesis
N	3	0.13	Cell motility
Z	0	0	Cytoskeleton
W	0	0	Extracellular structures
U	27	1.16	Intracellular trafficking and secretion, and vesicular transport
O	66	2.83	Posttranslational modification, protein turnover, chaperones
C	76	3.26	Energy production and conversion
G	78	3.34	Carbohydrate transport and metabolism
E	112	4.8	Amino acid transport and metabolism
F	52	2.23	Nucleotide transport and metabolism
H	91	3.9	Coenzyme transport and metabolism
I	42	1.8	Lipid transport and metabolism
P	82	3.51	Inorganic ion transport and metabolism
Q	17	0.73	Secondary metabolites biosynthesis, transport and catabolism
R	150	6.43	General function prediction only
S	89	3.81	Function unknown
-	949	40.48	Not in COGs

^aThe total is based on the total number of protein coding genes in the annotated genome.



are protein-coding genes, six are pseudogenes, and nine are RNA genes. The percentage of the protein-coding genes that were assigned a putative function is 47.17%. The distribution of genes into COGs functional categories is presented in Table 4. One CRISPR motif was also detected.

Conclusions

Prior to this report only a single genome sequence was available for *O. rhinotracheale*, from the type strain LMG 9086, and no corresponding analysis of an *O. rhinotracheale* genome has been published. Examination of the aligned genomes of these isolates revealed that rearrangements and inversions are the major distinguishing features. Relative to LMG 9086, the genome of H06-030791 contains a single rearrangement of ~31 Kb, a single inversion of ~17 Kb and three regions that are both inverted and rearranged, varying from ~59-354 Kb each, many with a transposase or transposon present at one terminus. Thus, mobile elements may play a role in shaping genome structure and evolution of *O. rhinotracheale*. Within one of the inverted and rearranged segments of H06-030791 is an apparent deletion of ~37 Kb found in LMG 9086,

comprised primarily of CDSs annotated as hypothetical proteins but also including a holin family protein, an ATP-dependent serine protease, a helix-turn-helix protein and several phage-related proteins. Owing to gaps in the H06-030791 genome, the putative deletion requires confirmation but it does lie well within the boundaries of the contig in which it is found and adjacent sequences are syntenous with the LMG 9086 genome. Also within the same rearranged/inverted region is an insertion in H06-030791 with five predicted CDSs, four annotated as hypothetical proteins and one as a multidrug ABC transporter.

Notable phenotypes associated with H06-030791 but not the type strain include β -hemolytic activity [12] and the ability to grow in the presence of an iron chelator [11]. Only three CDSs whose annotations suggest a function in hemolytic activity were apparent in H06-030791. Identical or nearly identical homologs were found in the LMG 9086 genome. One additional CDS annotated in LMG 9086 as a hemolysin was also found in H06-030791, identical in sequence but annotated there as a glycerol acyltransferase. Among 15 CDSs collectively found in H06-030791 and LMG 9086 whose annotations suggest a

role in iron acquisition or transport, only one was found to have considerable sequence divergence. The integral membrane protein and ferrous iron transporter FeoB is predicted to be identical in both isolates over the N-terminal 395 amino acids but only 94.7% identical over the C-terminal 301 amino acids. Motifs found within the divergent region of the protein include a ferrous iron transport protein B C terminus (PF07664.7) flanked by two gate nucleoside recognition domains (PF07670.9). As these are believed to comprise the membrane pore region, sequence heterogeneity may perhaps affect the specificity of transport. Other homologs in H06-030791 and LMG 9086 with obvious sequence divergence include several annotated as hypothetical proteins, a transcriptional regulator/sugar kinase with a highly divergent stretch of ~50 bp, a Crp/Fnr family transcriptional regulator with nearly all amino acid substitutions in the cyclic nucleotide binding domain (PF00027.24) of the predicted protein and a PAO141 family polyphosphate kinase 2, with substitutions concentrated in the polyphosphate kinase 2 domain (PF03976.9).

The genome sequence of H06-030791, together with those of the type strain and an additional, recently sequenced isolate [38] will provide a framework for future investigations designed to elucidate the genetic basis of virulence in *O. rhinotracheale* and for understanding genome structure and evolution.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EZ participated in genome sequencing and drafted the original manuscript. DB directed genome sequence assembly and bioinformatics analyses. WB participated in genome sequencing and post-sequencing analyses. LT conceived of the study and participated in genome sequencing. KR participated in post-sequencing analysis and revised the manuscript. All authors read and approved the final manuscript.

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