High quality permanent draft genome sequence of *Phaseolibacter flectens* ATCC 12775<sup>T</sup>, a plant pathogen of French bean pods

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**Abstract**

*Phaseolibacter flectens* strain ATCC 12775<sup>T</sup> (Halpern et al., *Int J Syst Evol Microbiol* 63:268–273, 2013) is a Gram-negative, rod shaped, motile, aerobic, chemoorganotroph bacterium. *Ph. flectens* is a plant-pathogenic bacterium on pods of French bean and was first identified by Johnson (1956) as *Pseudomonas flectens*. After its phylogenetic position was reexamined, *Pseudomonas flectens* was transferred to the family *Enterobacteriaceae* as *Phaseolibacter flectens* gen. nov., comb. nov. Here we describe the features of this organism, together with the draft genome sequence and annotation. The DNA GC content is 44.34 mol%. The chromosome length is 2,748,442 bp. It encodes 2,437 proteins and 89 RNA genes. *Ph. flectens* genome is part of the Genomic Encyclopedia of Type Strains, Phase I: the one thousand microbial genomes study.

**Keywords:** *Phaseolibacter flectens*, *Enterobacteriaceae*, plant pathogen, French bean pod, *Phaseolus vulgaris*

**Introduction**

*Phaseolibacter flectens* ATCC 12775<sup>T</sup> (= CFBP 3281<sup>T</sup>, ICMP 745<sup>T</sup>, LMG 2187<sup>T</sup>, NCPPB 539<sup>T</sup>), was isolated from infected French bean (*Phaseolus vulgaris*) pods in Queensland, Australia by Johnson (1956) [1]. Johnson, identified strain ATCC 12775<sup>T</sup> as *Pseudomonas flectens* [1], however, 29 years later, De Vos et al. [2] argued, that *Ps. flectens* Johnson (1956) does not belong to the genus *Pseudomonas* and thus should be removed from this genus. Anzai et al. [3] demonstrated that *Ps. flectens* should be included in the cluster of the *Enterobacteriaceae* family [4]. Recently, Halpern et al. [5], reclassified the species *Ps. flectens* Johnson 1956 as the type species of a novel genus *Phaseolibacter* in the family *Enterobacteriaceae*, as *Phaseolibacter flectens* gen. nov., comb. nov. [5]. Currently, the *Enterobacteriaceae* family comprises more than 60 different genera. Species belonging to this family exist in diverse environments such as water, terrestrial habitats, human, animals, insects or plants [4].

Johnson [1], studied a disease which caused blighting and twisting of French bean pods. He isolated strain ATCC 12775<sup>T</sup> along with other strains that he identified as the same species from the diseased plants and proved that by inoculating healthy bean pods with pure culture of strain ATCC 12775<sup>T</sup>, the pods became twisted. The fact that the infection with *Ph. flectens* was confined to the pods, suggested that the introduction of the bacteria to the crop, took place after the flowering [1]. Johnson [1] demonstrated in experiments that were carried out in the laboratory and in a greenhouse, that bean thrips (*Taeniothrips nigricornis*), which are tiny, slender insects that feed on pollen and floral tissue, transmitted this plant pathogenic bacterium between the crop plants [1].

Here we describe a summary classification and a set of the features of the plant pathogenic bacterium *Ph. flectens*,...
together with the permanent draft genome sequence description and annotation of the type strain (ATCC 12775\textsuperscript{T}).

**Organism information**

**Classification and features**

*Ph. flectens* strain ATCC 12775\textsuperscript{T} share typical characteristics of *Enterobacteriaceae* members such as: Gram negative, facultative anaerobic, chemoheterotrophic rod, positive for catalase and glucose fermentation and negative for oxidase [5] (Table 1). The phylogenetic tree based on the 16S rRNA also supports the fact that strain ATCC 12775\textsuperscript{T} is a member of the family *Enterobacteriaceae* (Fig. 1), as was already suggested by Anzai et al. [3]. *Ph. flectens* is the type species of the genus *Phaseolibacter*, which currently comprises only one species [5].

Cells of *Ph. flectens* strain ATCC 12775\textsuperscript{T} are motile rods by means of one or two flagella, measuring 0.5–0.8 \(\mu\)m in width and 1.2–2.3 \(\mu\)m in length (Fig. 2). When cells are grown on LB or R2A agar media for 48 h, colonies are 1 mm diameter, however, when cells are grown on the same media supplemented with sucrose, colonies are 3–5 mm diameter, produce huge amount of mucus, smooth, foggy and grayish white colored and motility is not observed. Growth is observed under anaerobic conditions [5]. Grows at 4–44 °C (optimum, 28–30 °C), with 0–60 % sucrose (optimum, 10–25 % sucrose) (Table 1). Growth is observed on MacConkey agar. D-glucose, sucrose, D-melibiose, glycerol, D-fructose are fermented; acetoin is produced; \(\text{H}_2\text{S}\) and indole are not produced; gelatin and urea are not hydrolyzed; citrate is not utilized; nitrate is reduced to nitrogen. L-arabinose, D-manitol, inositol, sorbitol, rhamnose,

| MIGS ID | Property | Term | Evidence code\(^a\) |
|---------|----------|------|---------------------|
| MIGS-6 | Habitat | Pods of French bean | TAS [5] |
| MIGS-6.3 | Salinity | Unknown | NAS |
| MIGS-22 | Oxygen requirement | Facultative anaerobic | TAS [5] |
| MIGS-15 | Biotic relationship | Plant host-associated | TAS [5] |
| MIGS-14 | Pathogenicity | Plant pathogen | TAS [1] |
| MIGS-4 | Geographic location | Australia, Queensland | TAS [1] |
| MIGS-5 | Sample collection | 1956 | TAS [1] |
| MIGS-4.1 | Latitude | Unknown | NAS |
| MIGS-4.2 | Longitude | Unknown | NAS |
| MIGS-4.4 | Altitude | Unknown | NAS |

\(^a\) Evidence codes - IDA Inferred from Direct Assay, 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 [32]
and amygdalin are not fermented. Tryptophane deaminase activity is present; β-galactosidase, arginine dihydrolase, lysine and ornithine decarboxylases activities are absent [5].

Chemotaxonomic data
The major fatty acids are: C\textsubscript{16:0}; Summed feature 2 (one or more of C\textsubscript{14:0} 3-OH, iso-C\textsubscript{16:1} I and unknown ECL 10.928) and Summed feature 3 (C\textsubscript{16:1}ω\textsubscript{7}c and/or iso-C\textsubscript{15:0} 2-OH) [2]. Minor fatty acids are: unknown 13.957; C\textsubscript{17:0} cyclo; C\textsubscript{18:1}ω\textsubscript{7}c; C\textsubscript{12:0}; C\textsubscript{14:0} 2-OH and C\textsubscript{14:0} [5].

Genome sequencing information
Genome project history
This organism was selected for sequencing based on its phylogenetic position [6, 7] and is part of the study Genomic Encyclopedia of Type Strains, Phase I: the one thousand microbial genomes project [8]. The goal of the KMG-I study is to increase the coverage of sequenced reference microbial genomes [9]. The project is registered in the Genomes OnLine Database [10] and the permanent draft genome sequence is deposited in GenBank. Draft sequencing and assembly were performed at the DOE Joint Genome Institute (jgi.doe.gov) using state of the art sequencing technology [11]. A summary of the project information is shown in Table 2.

Growth conditions and genomic DNA preparation
Ph. flectens strain ATCC 12775\textsuperscript{T} was grown in the appropriate medium as recommended on the web pages of the collection (Nutrient agar or broth). The purity of the culture was determined by growth on general maintenance media. Cells were harvested by centrifugation and genomic DNA was extracted from lysozyme-treated cells.
using cetyltrimethyl ammonium bromide and phenol-chloroform. The purity, quality and size of the bulk genomic DNA preparation was assessed according to DOE-JGI guidelines. Amplification and partial sequencing of the 16S rRNA gene confirmed the identity of strain 12775T.

**Genome sequencing and assembly**

The draft genome of *Ph. flectens* was generated at the DOE Joint genome Institute (JGI) using the Illumina technology [12]. An Illumina std. shotgun library was constructed and sequenced using the Illumina HiSeq 2000 platform which generated 18,689,832 reads totaling 2,803.5 Mb. All general aspects of library construction and sequencing performed at the JGI can be found at the JGI website (jgi.doe.gov). All raw Illumina sequence data was passed through DUK, a filtering program developed at JGI, which removes known Illumina sequencing and library preparation artifacts (Mingkun L, Copeland A, Han J. DUK, unpublished, 2011). Following steps were then performed for assembly: (1). filtered Illumina reads were assembled using Velvet [13], (2). 1–3 kb simulated paired end reads were created from Velvet contigs using wgsim (https://github.com/lh3/wgsim), (3). Illumina reads were assembled with simulated read pairs using Allpaths–LG [14]. Parameters for assembly steps were: (1). Velvet (velveth: 63 –shortPaired and velvetg: –very clean yes –exportFiltered yes –min contig lgth 500 –scaffolding no –cov cutoff 10) (2). wgsim (–e 0 –1 100 –2 100 –r 0 –R 0 –X 0) (3). Allpaths–LG (PrepareAllpathsInputs: PHRED 64 = 1 PLOIDY = 1 FRAG COVERAGE = 125 JUMP COVERAGE = 25 LONG JUMP COV = 50, RunAllpathsLG: THREADS = 8 RUN = std shredpairs TARGETS = standard VAPI WARN ONLY = True OVERWRITE = True). The final draft assembly contained 29 contigs in 26 scaffolds, totalling 2.7 Mb in size. The final assembly was based on 1,500.0 MB of Illumina data.

**Genome annotation**

The assembled sequence was annotated using the JGI prokaryotic annotation pipeline [15] and was further reviewed using the Integrated Microbial Genomes—Expert Review platform [16]. Genes were identified using Prodigal [17]. CRISPR elements were detected using CRT [18] and PILER-CR [19]. The final annotated genome is available from the Integrated Microbial Genome system [20].

**Genome properties**

The assembly of the draft genome sequence consists of 26 scaffolds amounting to 2,748,442 bp, and the G + C content is 44.34 % (Table 3, Additional file 1: Table S1). Of the 2,526 genes predicted, 2,437 were protein-coding genes, and 89 RNAs. The majority of the protein-coding genes (81.2 %) were assigned a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

**Insights from the genome sequence**

*Ph. flectens* was isolated from pods of diseased French bean plants. The genome of *Ph. flectens* strain ATCC 12775T reveals the presence of virulence associated genes which demonstrate that indeed, this species has the potential to attack plant tissues. Salmonellla-Shigella invasin protein C (IpaC SipC) gene is present in the genome of *Ph. flectens* and represents a family of proteins associated with bacterial type III secretion systems, which are

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**Table 2** Genome sequencing project information

| MIGS ID | Property | Term |
|---------|----------|------|
| MIGS 31.1 | Finishing quality | Level 2: high-quality draft |
| MIGS 28 | Libraries used | Illumina std shotgun library |
| MIGS 29 | Sequencing platforms | Illumina HiSeq 2000, Illumina HiSeq 2500 |
| MIGS 31.2 | Fold coverage | 561X |
| MIGS 30 | Assemblers | Velvet (v. 1.1.04), ALLPATHS–LG (v. r42328) |
| MIGS 32 | Gene calling method | Prodigal 2.5 |
| Locus tag | L871 |
| Genbank ID | JAE00000000 |
| Genbank date of release | 23-JAN-2014 |
| GOLD ID | Gp0032039 |
| BIOPROJECT | PRJNA204094 |
| MIGS-13 | Source material identifier | ATCC 12775 |
| Project relevance | GEBA-KMG, tree of life |
injection machines for virulence factors into host cell cytoplasm. A heat labile enterotoxin alpha chain that belongs to the ADP-ribosylation superfamily, is also present in the *Ph. flectens* genome. Five genes in the genome of *Ph. flectens* encode the virulence factor hemolysin which has a lytic activity on eukaryotic cells. These genes are: hemolysin activationSECRETION protein (two copies); hemolysin-coregulated protein; phospholipase/lecithinase/hemolysin; hemolysins and related proteins containing CBS domains and putative hemolysin. Two copies of a gene encoding filamentous hemagglutinin family N-terminal domain are encoded in the genome of ATCC 12775T, representing another virulence potential of this bacterium. Filamentous hemagglutinin-like adhesins are virulence factors in both plant and animal pathogens and have a role in the attachment, aggregation and cell killing [21]. Another feature of bacterial phytopathogenesis is the secretion of pectinolytic enzymes by microorganisms [22]. Pectate lyase (two copies) is found in the genome, demonstrating the potential of this species to degrade the pectic components of the plant cell wall.

The potential of *Ph. flectens* to produce pili is evident from the presence of seven pili genes: prepilin-type N-terminal cleavage/methylation domain; P pilus assembly protein, pilin FimA (eight copies); P pilus assembly protein, chaperone PapC (two copies); P pilus assembly protein, chaperone PapD (three copies); P pilus assembly/Cpx signaling pathway, periplasmic inhibitor/zinc-resistance associated protein; Type II secretory pathway, ATPase PulE/Tfp pilus assembly pathway, ATPase PilB and CblD like pilus biogenesis initiator (two copies).

The presence of the gene for S-ribosylhomocysteine lyase LuxS indicates that *Ph. flectens* produces quorum-sensing autoinducer 2 (AI-2).

**Conclusions**

In the current study we characterized the genome of *Ph. flectens* strain ATCC 12775T, that was isolated from French bean pods in Queensland, Australia [1]. Strain ATCC 12775T is a plant pathogen that cause pod twist disease in French bean plants. The bacteria cause the destruction of immature bean pods, immediately after the flowering stage. The blighted pods wither and drop to the ground or remain hanging and become twisted. Bean thrips (*Taeniothrips nigricornis*), are the ones that probably transmit this plant pathogenic bacterium between the crop plants [1]. Genes indicating the potential of strain ATCC 12775T to cause plant disease were found in the bacterial genome. Among them were: injection machine for virulence factors into host cell cytoplasm (invasin protein C (IpaC_SipC)); heat labile enterotoxin; phospholipase/lecithinase/hemolysin which is capable of destroying the Eukaryotic cell membrane; filamentous hemagglutinin-like adhesins which have a role in the

### Table 3: Genome statistics

| Attribute                      | Value        | % of Total |
|--------------------------------|--------------|------------|
| Genome size (bp)               | 2,748,442    | 100.00     |
| DNA coding (bp)                | 2,272,995    | 82.70      |
| DNA G+C (bp)                   | 1,218,718    | 44.34      |
| DNA scaffolds                   | 26           | 100.00     |
| Total genes                    | 2,526        | 100.00     |
| Protein coding genes           | 2,437        | 96.48      |
| RNA genes                      | 89           | 3.52       |
| Pseudo genes                   | 0            | 0.00       |
| Genes in internal clusters     | 1,553        | 61.48      |
| Genes with function prediction | 2,051        | 81.20      |
| Genes assigned to COGs         | 1,800        | 71.26      |
| Genes with Pfam domains        | 2,103        | 83.25      |
| Genes with signal peptides     | 179          | 7.09       |
| Genes with transmembrane helices| 552          | 21.85      |
| CRISPR repeats                 | 1            |            |

### Table 4: Number of genes associated with the general COG functional categories

| Code | Value | % of Total | Description                                      |
|------|-------|------------|--------------------------------------------------|
| J    | 221   | 11.05      | Translation, ribosomal structure and biogenesis   |
| A    | 1     | 0.05       | RNA processing and modification                   |
| K    | 104   | 5.20       | Transcription                                     |
| L    | 112   | 5.60       | Replication, recombination and repair             |
| B    | 0     | 0.00       | Chromatin structure and dynamics                  |
| D    | 40    | 2.00       | Cell cycle control, cell division, chromosome partitioning |
| V    | 44    | 2.20       | Defense mechanisms                                |
| T    | 67    | 3.35       | Signal transduction mechanisms                    |
| M    | 188   | 9.40       | Cell wall/membrane biogenesis                     |
| N    | 34    | 1.70       | Cell motility                                     |
| U    | 69    | 3.45       | Intracellular trafficking, secretion and vesicular transport |
| O    | 92    | 4.60       | Posttranslational modification, protein turnover, chaperones |
| C    | 104   | 5.20       | Energy production and conversion                  |
| G    | 104   | 5.20       | Carbohydrate transport and metabolism             |
| E    | 190   | 9.50       | Amino acid transport and metabolism               |
| F    | 65    | 3.25       | Nucleotide transport and metabolism               |
| H    | 112   | 5.60       | Coenzyme transport and metabolism                 |
| I    | 75    | 3.75       | Lipid transport and metabolism                    |
| P    | 104   | 5.20       | Inorganic ion transport and metabolism            |
| Q    | 38    | 1.90       | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 96    | 4.80       | General function prediction only                  |
| S    | 95    | 4.75       | Function unknown                                  |
| -    | 726   | 28.74      | Not in COGs                                      |
attachment, aggregation and host cell killing [21] and pectate lyase that has the potential to degrade the pectic components of the plant cell wall [22].

Additional file

Additional file 1: Table S1. Scaffolds and contigs of Genomic DNA for Phaseolobacter Reactens ATCC 12775T (Topology: linear; Read depth: 1.00).

Abbreviations

KMG: One thousand microbial genomes; GEBA: Genomic encyclopedia of Bacteria and Archaea; MIGS: Minimum information about a genome sequence; TAS: Traceable; NAS: Non-traceable.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

YGA, II and MH characterized strain ATCC 12775T and transferred it from the genus Pseudomonas to Phaseolobacter gen. nov. YGA, II, MH, AL and NCK drafted the manuscript. AC, TBKR, MH, MP, MG, VM, TW and NCK sequenced, assembled and annotated the genome. All authors read and approved the final manuscript.

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