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GENOME ANNOUNCEMENT

Draft Genome Sequence of the Cyanide-Utilizing Bacterium *Pseudomonas fluorescens* Strain NCIMB 11764

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We report here the 6.97-Mb draft genome sequence of *Pseudomonas fluorescens* strain NCIMB 11764, which is capable of growth on cyanide as the sole nitrogen source. The draft genome sequence allowed the discovery of several genes implicated in enzymatic cyanide turnover and provided additional information contributing to a better understanding of this organism's unique cyanotrophic ability. This is the first sequenced genome of a cyanide-assimilating bacterium.

Pseudomonas fluorescens strain NCIMB 11764 was isolated in 1983 by Harris and Knowles (5) from enrichment culture-supplied potassium cyanide (KCN) as the sole nitrogen source. Cyanide is metabolized oxidatively by *P. fluorescens* 11764, yielding carbon dioxide and ammonia as metabolic products (3, 4, 6, 7), with the ammonia satisfying the nitrogen requirement for growth. The genomes of several well-characterized *P. fluorescens* strains (Pf01, Pf5, and SWB25) have been determined, but cyanide utilization (cyanotrophy) is not a phenotypic trait of any of those strains.

The *P. fluorescens* 11764 genome was sequenced by Eureka Genomics (Hercules, CA) by using the Illumina Genome Analyzer IIx from paired-end libraries (average insert size, 221 bp). The total number of reads was 16,174,118, with a total length of 841,054,136 bp. The quality of sequencing reads was determined with the FastQC program (version 0.10.0 [www.bioinformatics.babraham.ac.uk/projects/fastqc/]). The average length of reads was 51 bp, with a good range of quality values across all bases at each position. Accordingly, a sequencing coverage of about 120-fold was inferred. The reads were assembled with the SOAPdenovo software, version 1.04-linux-32 (8), using a k-mer size of 31 bp. In total, 831 contigs with lengths greater than 200 bp (N50 = 15,804 bp) were obtained, which accounts for 96% of the total hypothesized genome content. A pseudochromosome was generated by ordering contigs based upon alignments with the reference genome of *P. fluorescens* strain Pf0-1 using BLAST, version 2.2.23 (1). The Rapid Annotation using Subsystem Technology (RAST) (2) server version 4.0 was used to predict and annotate the genes on the draft genome. The SEED viewer version 2.0 (10) was used to categorize predicted genes into functional subsystems. tRNAs were predicted using the tRNAscanSE program, version 1.3 (9).

The total assembled draft genome was 6,966,196 bp long, with a GC content of 59%. This is in close approximation to that of other *P. fluorescens* genome sizes (12, 13). In the *P. fluorescens* 11764 draft genome, 40 tRNA and 6,307 protein-coding genes were predicted. The most abundant proportions of predicted metabolic genes were those related to biosynthesis and degradation of amino acids, carbohydrate metabolism, cofactor metabolism, and pigment biosynthesis. This is in accordance with the expected metabolism of *P. fluorescens* species commonly encountered as soil and plant inhabitants. Additionally, a high proportion of genes linked to RNA/DNA metabolism, regulation, and cell signaling were found, which is consistent with the complexity of molecular regulation in larger genomes (12, 14).

Previous studies (3, 4) of the metabolic basis of cyanotrophy in *P. fluorescens* 11764 pointed to the involvement of several enzymes (NADH oxidase [Nox], NADH peroxidase [Npx], cyanide dihydratase [CynD], and carbonic anhydrase [CA]) in oxidative cyanide turnover to ammonia, with the latter then incorporated into biomolecules. The *P. fluorescens* 11764 genome was searched for genes encoding these enzymes, and in total, four Nox, two Npx, and six CA genes were identified. Four genes with homology to CynD, a member of the nitrilase superfamily (11), were uncovered.

Nucleotide sequence accession number. The draft genome sequence of *P. fluorescens* 11764 has been deposited at DDBJ/EMBL/GenBank under accession number ALWP00000000.

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REFERENCES

1. **Altschul S, et al.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**:3389–3402.
2. **Aziz RK, et al.** 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics **9**:75. doi:10.1186/1471-2164-9-75.
3. **Fernandez RF, Dolghih E, Kunz DA.** 2004. Enzymatic assimilation of cyanide via pterin-dependent oxygenolytic cleavage to ammonia and formate in *Pseudomonas fluorescens* NCIMB 11764. Appl. Environ. Microbiol. **70**:121–128.
4. **Fernandez RF, Kunz DA.** 2005. Bacterial cyanide oxygenase is a suite of enzymes catalyzing the scavenging and adventitious utilization of cyanide as a nitrogenous growth substrate. J. Bacteriol. **187**:6396–6402.
5. **Harris R, Knowles CJ.** 1983. Isolation and growth of a *Pseudomonas* species that utilizes cyanide as a source of nitrogen. J. Gen. Microbiol. **129**:1005–1011.
6. **Harris R, Knowles CJ.** 1983. The conversion of cyanide to ammonia by extracts of a strain of *Pseudomonas fluorescens* that utilizes cyanide as a source of nitrogen for growth. FEMS Microbiol. Lett. **20**:337–341.
7. **Kunz DA, Nagappan O, Silva-Avalos J, Delong GT.** 1992. Utilization of cyanide as a nitrogenous substrate by *Pseudomons fluorescens* NCIMB

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11764: evidence for multiple pathways of metabolic conversion. Appl. Environ. Microbiol. **58**:2022–2029.
8. **Li R, et al.** 2010. De novo assembly of human genomes with massively parallel short read sequencing. Genome Res. **20**:265–272.
9. **Lowe T, Eddy S.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**:955–964.
10. **Overbeek R, et al.** 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res. **33**:5691–5702.
11. **Pace HC, Brenner C.** 2001. The nitrilase superfamily: classification, structure and function. Genome Biol. **2**:1–9.
12. **Paulsen IT, et al.** 2005. Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. Nat. Biotechnol. **23**:873–878.
13. **Silby MW, et al.** 2009. Genomic and genetic analyses of diversity and plant interactions of *Pseudomonas fluorescens.* Genome Biol. **10**:R51. doi: 10.1186/gb-2009-10-5-r51.
14. **Silby MW, Winstanley C, Godfrey SA, Levy SB, Jackson RW.** 2011. *Pseudomonas* genomes: diverse and adaptable. FEMS Microbiol. Rev. **35**: 652–680.