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GENOME ANNOUNCEMENT

Genome Sequence of *Stenotrophomonas maltophilia* PML168, Which Displays Baeyer-Villiger Monooxygenase Activity

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Stenotrophomonas maltophilia PML168 was isolated from Wembury Beach on the English Coast from a rock pool following growth and selection on agar plates. Here we present the permanent draft genome sequence, which has allowed prediction of function for several genes encoding enzymes relevant to industrial biotechnology, including a novel flavoprotein monooxygenase.

Stenotrophomonas maltophilia strain PML168 was sampled and isolated from a rock pool found on Wembury Beach on the English Coast (50°18'N, 4°05'W). As part of an isolation and screening program of coastal microbial communities, strain PML168 was isolated following the scraping of biomass from a rock surface and its transfer to, and clonal isolation on, R2A agar (Difco) at 15°C. The strain is currently maintained in the PML Microbe Collection. Initial phylogenetic analysis of a 402-bp 16S rRNA gene fragment sequence placed PML168 as a member of the Xanthomonadaceae within the Gammaproteobacteria group. In a screen for industrially relevant enzymatic activities, as part of an in-house biocatalysis discovery program (5, 9), PML168 displayed general alkaline phosphomonoesterase, alkaline phosphodiesterase, carboxyesterase, epoxide hydrolase, halocarboxylic acid dehalogenase, EC 1.1 (CH-OH) and EC 1.3 (CH-CH) dehydrogenase, peroxidase, laccase, and lactone hydrolase activities. In addition, PML168 also performed the Baeyer-Villiger oxidation of the substrate 3-acetyl indole (1, 11).

Genomic DNA was prepared at Plymouth Marine Laboratory and sent to the GenePool Genomics and Bioinformatics Facility at the University of Edinburgh for sequencing and assembly. A paired-end sequencing library was prepared using Illumina sample preparation kit v1 and sequenced using the Illumina genome analyzer II (GAII) platform. A total of 8,218,403 paired-end, 50base reads were used for *de novo* assembly using Velvet 0.7.31 (12), resulting in 48 scaffolds and 562 contigs (45 of >1 kbp) comprising 4,439,730 bp, with a maximum contig length of 582,338 bp and 7 contigs with an N_{50} of 243,851 bp. The assembled contigs were loaded into IMG-ER for gene prediction and annotation prior to submission to GenBank (7). The draft genome of PML168 has a G+C content of 67.1% and contains 4,067 predicted coding sequences (CDSs; average length, 992 bp), 6 rRNA genes, and 59 tRNAs, all of which comprise approximately 89.4% of the genome.

A putative function could be predicted for 3,101 (76.25%) of the CDSs, whereas 896 of the CDSs were annotated as hypothetical proteins. Full 16S rRNA sequence data confirmed its identity as *Stenotrophomonas maltophilia*, with 100% identity to *Stenotrophomonas maltophilia* LMG10857 (3). The sequencing of PML168 allowed the identification of CDSs potentially responsible for all of the activities observed in the previous enzymatic laboratory screens (see above). Of particular note, a CDS encoding an interesting 357-amino-acid (38.6-kDa) flavin-dependent monooxygenase (FMO) was identified. The sequence contains two Rossmann fold motifs (GXGXXG) and carries the FMO motif (FXGX XXHXXY) (2, 8, 10). It has been shown that some FMOs possess the ability to catalyze Baeyer-Villiger reactions (6). The whole-cell Baeyer-Villiger monooxygenase activity of this strain has been described in detail (11). Biochemical characterization of the FMO has shown no 3-acetyl indole oxidation activity; however, it does catalyze the oxidation of sulfides and also the regioselective Baeyer-Villiger oxidation of bicyclo[3.2.0]hept-2-en-6-one (4). The biotechnological application of this enzyme is currently being evaluated.

Nucleotide sequence accession numbers. The nucleotide sequence for the draft genome sequence was deposited in EMBL under accession numbers CAJH01000001 to CAJH01000097.

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REFERENCES

- Baeyer A, Villiger V. 1899. Einwirkung des Caro'schen Reagens auf Ketone. Berichte der deutschen chemischen Gesellschaft 32:3625–3633.
- Fraaije MW, Kamerbeek NM, van Berkel WJH, Janssen DB. 2002. Identification of a Baeyer-Villiger monooxygenase sequence motif. FEBS Lett. 518:43–47.

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- Hauben L, Vauterin L, Moore ERB, Hoste B, Swings J. 1999. Genomic diversity of the genus Stenotrophomonas. Int. J. Syst. Bacteriol. 49:1749– 1760.
- Jensen CN, et al. 2012. A flavoprotein monooxygenase that catalyses a Baeyer–Villiger reaction and thioether oxidation using NADH as the nicotinamide cofactor. Chembiochem 13:872–878.
- Joint I, Mühling M, Querellou J. 2010. Culturing marine bacteria—an essential prerequisite for biodiscovery. Microb. Biotechnol. 3:564–575.
- Lai WG, Farah N, Moniz GA, Wong YN. 2011. A Baeyer-Villiger oxidation specifically catalyzed by human flavin-containing monooxygenase 5. Drug Metab. Dispos. 39:61–70.
- Markowitz VM, et al. 2009. IMG ER: a system for microbial genome annotation expert review and curation. Bioinformatics 25:2271–2278.

- Rao ST, Rossmann MG. 1973. Comparison of super-secondary structures in proteins. J. Mol. Biol. 76:241–256.
- 9. Reid E, et al. 2011. Coccolithophores: functional biodiversity, enzymes and bioprospecting. Mar. Drugs 9:586–602.
- van Berkel WJH, Kamerbeek NM, Fraaije MW. 2006. Flavoprotein monooxygenases, a diverse class of oxidative biocatalysts. J. Biotechnol. 124:670-689.
- 11. Willetts A, Joint I, Gilbert JA, Trimble W, Mühling M. 2012. Isolation and initial characterization of a novel type of Baeyer-Villiger monooxygenase activity from a marine microorganism. Microb. Biotechnol. 2012: 1751–7915.
- 12. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 18:821–829.