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Genome Sequences of the Lignin-Degrading *Pseudomonas* sp. Strain YS-1p and *Rhizobium* sp. Strain YS-1r Isolated from Decaying Wood

Madhu Prabhakaran,[a] Matthew B. Couger,[a] Colin A. Jackson,[a] Tyler Weirick,[b] Babu Z. Fathepure[a]

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, Oklahoma, USA[a]; Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, Oklahoma, USA[b]

***Pseudomonas* sp. strain YS-1p and *Rhizobium* sp. strain YS-1r were isolated from a lignin-degrading enrichment culture. The isolates degraded lignin-derived monomers, dimers, alkali lignin, and, to a smaller extent (3% to 5%), lignin in switch grass and alfalfa. Genome analysis revealed the presence of a variety of lignin-degrading genes.**

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Address correspondence to Babu Z. Fathepure, babu.fathepure@okstate.edu.

Lignin is the second most abundant terrestrial polymer after cellulose and is found in most terrestrial plants in significant amounts ranging from 15% to 40% of dry weight (1). Lignin cross-links with hemicellulose and cellulose and resists microbial attack forming a major roadblock for effective saccharification of plant biomass (2, 3). To date, studies have focused primarily on fungal ability to breakdown lignin as they produce high levels of efficient enzymes that degrade lignin (4–6). However, in recent years, emphasis has shifted toward understanding the bacterial role in lignin degradation because bacteria are widespread and can offer novel catalytic pathways for the development of efficient pretreatment technologies (7–11). The primary goals of this study were to isolate and characterize bacteria that degrade lignin as the sole source of carbon. We have isolated a *Pseudomonas* sp. strain YS-1p and *Rhizobium* sp. strain YS-1r that degrade lignin and lignin-like compounds as the sole sources of carbon from an enrichment developed from a decaying wood from a thermal pond at Yellowstone National Park, WY. To obtain insights into the lignin-degradation capacity of strains, YS-1p and YS-1r, draft genomes were analyzed.

Genomes of the strain YS-1p and strain YS-1r were sequenced using the Illumina miSeq platform using 250×2 paired-end reads. Sequence libraries were created using the standard Illumina Truseq library creation kit with an average insert size of 550 bp. Quality filtered sequence data were subsampled to ~150× coverage and assembled with the short read de Brujin graph (12) assembly program Velvet (13) using a k-mer value of 55. This assembly produced 103 contigs with a contig N_{50} of 199 kb, and total 6,380,264 bp for strain YS-1p and similarly the assembly produced 84 contigs with a contig N_{50} of 186 kb, and a total 6,385,152 bp for strain YS-1r. Genes were identified using the Prodigal algorithm (14). From the resulting assemblies, 5,796 gene models were produced for strain YS-1p and 5,709 for YS-1r. All proteins sequences were functionally annotated using a combination of NCBI Blast (15) and HMMER 3.0 (16) against the PFAM 27.0 database (17).

Genome analysis revealed that both organisms contain genes that code for enzymes needed for the degradation lignin and lignin-derived aromatic compounds including laccase, Dyp-peroxidase, beta-etherase, vanillate *O*-demethylase, feruloyl esterase, carboxyl esterase, cytochrome P450, and chloroperoxidase. Also, genes for aromatic ring-oxidation and ring-cleavage including phenol 2-monooxygenase, 4-hydroxybenzoate 3-monooxygenase, catechol 2,3-dioxygenase, protocatechuate 3,4-dioxygenase, and gentisate 1,2-dioxygenase were detected. Our laboratory experiments revealed that both isolates can degrade a variety of lignin monomers and dimers as the sole sources of carbon. Also, the organisms utilized alkali lignin (Sigma-Aldrich) and lignin in switch grass and alfalfa as the growth substrates. These observations suggest that bacteria can play important roles in plant biomass conversion technologies.

Nucleotide sequence accession numbers. The draft genome sequences of YS-1p and strain YS-1r have been deposited at DDBJ/EMBL/GenBank under the accession numbers JPYP00000000 and JPYQ00000000, respectively. The versions described here are versions JPYP01000000 and JPYQ01000000, respectively.

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REFERENCES

1. **Ragauskas AJ, Beckham GT, Biddy MJ, Chandra R, Chen F, Davis MF, Davison BH, Dixon RA, Gilna P, Keller M, Langan P, Naskar AK, Saddler JN, Tschaplinski TJ, Tuskan GA, Wyman CE.** 2014. Lignin valorization: improving lignin processing in the Biorefinery. Science **344:** 1246843. http://dx.doi.org/10.1126/science.1246843.
2. **Pothiraj C, Kanmani P, Balaji P.** 2006. Bioconversion of lignocellulose materials. Mycobiology **34:**159–165. http://dx.doi.org/10.4489/MYCO.2006.34.4.159.
3. **Ruiz-Dueñas FJ, Martínez ÁT.** 2009. Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. Microb Biotechnol **2:**164–177. http://dx.doi.org/10.1111/j.1751-7915.2008.00078.x.
4. **Kirk TK, Farrell RL.** 1987. Enzymatic "combustion": the microbial degradation of lignin. Annu Rev Microbiol **41:**465–505. http://dx.doi.org/10.1146/annurev.mi.41.100187.002341.
5. **Tien M, Kirk TK.** 1984. Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization, and catalytic properties of a

unique H_2O_2-requiring oxygenase. Proc Natl Acad Sci U S A **81:** 2280–2284. http://dx.doi.org/10.1073/pnas.81.8.2280.
6. **Wong DWS.** 2009. Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol **157:**174–209. http://dx.doi.org/10.1007/s12010-008-8279-z.
7. **Bandounas L, Wierckx NJP, de Winde JH, Ruijssenaars HJ.** 2011. Isolation and characterization of novel bacterial strains exhibiting ligninolytic potential. BMC Biotechnol **11:**94. http://dx.doi.org/10.1186/1472-6750-11-94.
8. **Bugg TDH, Ahmad M, Hardiman EM, Singh R.** 2011. The emerging role for bacteria in lignin degradation and bio-product formation. Curr Opin Biotechnol **22:**394–400. http://dx.doi.org/10.1016/j.copbio.2010.10.009.
9. **Masai E, Katayama Y, Fukuda M.** 2007. Genetic and biochemical investigations on bacterial catabolic pathways for lignin-derived aromatic compounds. Biosci Biotechnol Biochem **71:**1–15. http://dx.doi.org/10.1271/bbb.60437.
10. **Ramachandra M, Crawford DL, Hertel G.** 1988. Characterization of an extracellular lignin peroxidase of the lignocellulolytic actinomycete *Streptomyces viridosporus*. Appl Environ Microbiol **54:**3057–3063.
11. **Vicuña R.** 1988. Bacterial degradation of lignin. Enzyme Microb Technol **10:**646–655. http://dx.doi.org/10.1016/0141-0229(88)90055-5.
12. **Compeau PE, Pevzner PA, Tesler G.** 2011. How to apply de Bruijn graphs to genome assembly. Nat Biotechnol **29:**987–991. http://dx.doi.org/10.1038/nbt.2023.
13. **Zerbino DR, Birney E.** 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res **18:**821–829. http://dx.doi.org/10.1101/gr.074492.107.
14. **Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ.** 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics **11:**119. http://dx.doi.org/10.1186/1471-2105-11-119.
15. **Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL.** 2009. BLAST+: architecture and applications. BMC Bioinformatics **10:**421. http://dx.doi.org/10.1186/1471-2105-10-421.
16. **Eddy SR.** 2011. Accelerated profile HMM searches. PLoS Comput Biol **7:**e1002195. http://dx.doi.org/10.1371/journal.pcbi.1002195.
17. **Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer ELL, Tate J, Punta M.** 2014. Pfam: the protein families database. Nucleic Acids Res **42:**D222–D230. http://dx.doi.org/10.1093/nar/gkt1223.