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# Draft genome sequence of humic substances-degrading *Pseudomonas kribbensis* CHA-19 from temperate forest soil

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# 중위도 산림토양에서 분리한 부식질 분해능이 있는 *Pseudomonas kribbensis* CHA-19의 유전체 염기서열 초안

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*Pseudomonas kribbensis* CHA-19 was isolated from a temperate forest soil (mid latitude) in New Jersey, USA, for its ability to degrade humic acids, a main component of humic substances (HS), and subsequently confirmed to be able to decolorize lignin (a surrogate for HS) and catabolize lignin-derived ferulic and vanillic acids. The draft genome sequence of CHA-19 was analyzed to discover the putative genes for depolymerization of polymeric HS (e.g., dye-decolorizing peroxidases and laccase-like multicopper oxidases) and catabolic degradation of HS-derived small aromatics (e.g., vanillate *O*-demethylase and biphenyl 2,3-dioxygenase). The genes for degradative activity were used to propose a HS degradation pathway of soil bacteria.

**Keywords:** catabolic pathway, degradative enzyme, humic acids, soil bacteria

Humic substances (HS) are a natural complex heteropolymer, which are widely distributed in various cold, temperate, and tropical soils. HS and HS-derived compounds regulate the growth of plants and microorganisms through various and continuous interactions within soils (Grinhut *et al.*, 2011; Lipczynska-kochany, 2018). Owing to a structural similarity between lignin and HS, bacterial HS-degradative pathways were proposed based on previous studies for lignin degradation (Bugg *et al.*, 2011; Kamimura *et al.*, 2017; Kim *et al.*, 2018). It is assumed that HS are depolymerized by bacterial extracellular enzymes, such as dye-decolorizing peroxidases and laccase-like multi-copper oxidases, and the resulting HS-derived small aromatic compounds are uptaken into the cells and further catabolized.

A forest soil containing decaying plant material was sampled to study on the HS microbial degradation from New Jersey, USA, in September 2016. A bacterial strain (CHA-19) was isolated from the soil using an MSB minimal-agar plate owing to its ability to degrade humic acids (HA, Sigma-Aldrich; Cat. no. 53680). CHA-19 was able to decolorize lignin (Sigma-Aldrich; Cat. no. 370959) and catabolize lignin-derived mono-aromatics (ferulic and vanillic acids).

The analysis of 16S rRNA gene of CHA-19 (GenBank no. MK660005) showed that it was phylogenetically closest to *Pseudomonas kribbensis* 46-2$^T$ (99.93% similarity), *P. koreensis* Ps 9-14$^T$ (99.59% similarity) and *P. moraviensis* CCM 7280$^T$ (99.45% similarity). Genome sequencing of CHA-19 was performed at ChunLab, Inc. using the Illumina Miseq sequencing

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method and the sequence was assembled *de novo* into 34 contigs with SPAdes 3.10.1 (Bankevich *et al*., 2012). The average nucleotide identity (ANI) values between the type strains of *P. kribbensis*, *P. koreensis*, and *P. moraviensis* and CHA-19 were 95.88%, 88.82%, and 87.81%, respectively, by ChunLab TrueBac ID algorithm, and thus this strain was finally named *Pseudomonas kribbensis* CHA-19 (= KCTC 72262).

The draft genome sequence was approximately 6.4 Mb long with a G+C content of 60.6%. The resulting $N_{50}$ size of contigs was 413,591 bp and the total coverage over the genome was 297-fold. Following NCBI GenBank submission, the genes in draft genome sequence were annotated with NCBI Prokaryotic Genome Annotation Pipeline (PGAP) using best-placed reference protein set; GeneMarkS-2 method (Lomsadze *et al*., 2018). The genome annotation revealed 5,737 coding sequences (CDSs), 64 tRNA genes, and 4 rRNA genes (two for 5S, one for 16S, and one for 23S). Several putative HS-degradative genes were detected on the CHA-19 draft genome, which were used to propose a HS-degradation pathway by CHA-19 (Fig. 1): laccase-like multicopper oxidases [GenBank accession no. TFH77958 (*moxA*) and TFH78995], dye-decolorizing peroxidases [TFH80052 (*efeB*), TFH80975 (*yfeX*), and TFH81056 (*yfeX*)], biphenyl 2,3-dioxygenase [TFH78976 (*cntA*), TFH79968 (*hsaC*), and TFH80324 (*hcaE*)], 2,3-dihydroxybiphenyl-1,2-dioxygenase [TFH79858 (*hsaC*)], vanillate *O*-demethylase [TFH78866 (*vanB*) and TFH79337 (*vanA*)], protocatechuate 3,4-dioxygenase for *ortho*-ring cleavage [TFH82232 (*pcaH*) and TFH82233 (*pcaG*)], and catechol 1,2-dioxygenase for *ortho*-ring cleavage [TFH78177 (*catA*)].

## Nucleotide sequence accession number

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession SPDQ00000000. The version described in this paper is version SPDQ01000000 and consists of sequences SPDQ01000001-SPDQ01000034.

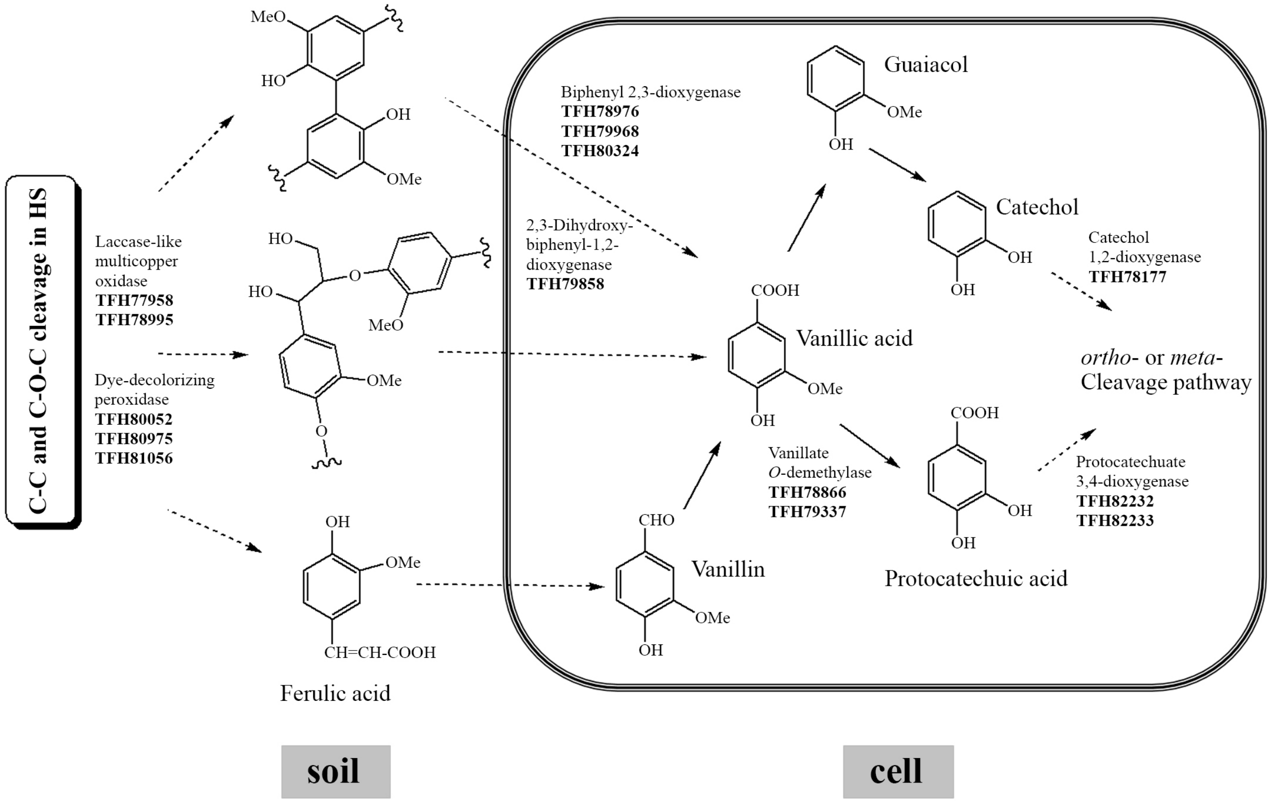

**Fig. 1. Proposed HS-degradative pathway by *Pseudomonas kribbensis* CHA-19.** Dotted and solid lines represent multi-step reactions by different enzymes and one-step reactions by one enzyme, respectively. GenBank accession numbers for putative enzymes catalyzing the corresponding reactions are shown next to the lines.

## 적 요

미국 뉴저지주 중위도 산림토양에서 부식산(천연 복합유기화합물인 부식질의 주요 구성성분) 분해능이 있는 세균 균주 *Pseudomonas kribbensis* CHA-19를 분리하였으며, 이후 또 다른 토양 유기물인 리그닌과 리그닌 유래의 페룰산(ferulic acid)과 바릴린산(vanillic acid)의 분해능을 확인하였다. 부식질 초기 저분자화 효소(예, dye-decolorizing peroxidase와 laccase-like multicopper oxidase)와 부식질 유래의 다양한 저분자 분해산물들을 분해하는 효소(예, vanillate *O*-demethylase와 biphenyl 2,3-dioxygenase)를 탐색하기 위해 CHA-19 게놈 염기서열을 분석하였다. 최종 확보한 효소유전자 정보는 토양 세균의 부식질 분해경로 제안에 사용되었다.

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## References

**Bankevich A, Nurk S, Antipov D, Gurevich A, Dvorkin M, Kulikov AS, Lesin V, Nikolenko S, Pham S, Prjibelski A, *et al.*** 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **19**, 455–477.

**Bugg TD, Ahmad M, Hardiman EM, and Rahmanpour R.** 2011. Pathways for degradation of lignin in bacteria and fungi. *Nat. Prod. Rep.* **28**, 1883–1896.

**Grinhut T, Hertkorn N, Schmitt-Kopplin P, Hadar Y, and Chen Y.** 2011. Mechanisms of humic acids degradation by white rot fungi explored using $^1$H NMR spectroscopy and FTICR mass spectrometry. *Environ. Sci. Technol.* **45**, 2748–2754.

**Kamimura N, Takahashi K, Mori K, Araki T, Fujita M, Higuchi Y, and Masai E.** 2017. Bacterial catabolism of lignin-derived aromatics: New findings in a recent decade: Update on bacterial lignin catabolism. *Environ. Microbiol. Rep.* **9**, 679–705.

**Kim D, Park HJ, Sul WJ, and Park H.** 2018. Transcriptome analysis of *Pseudomonas* sp. from subarctic tundra soil: pathway description and gene discovery for humic acids degradation. *Folia Microbiol. (Praha)* **63**, 315–323.

**Lipczynska-Kochany E.** 2018. Humic substances, their microbial interactions and effects on biological transformations of organic pollutants in water and soil: A review. *Chemosphere* **202**, 420–437.

**Lomsadze A, Gemayel K, Tang S, and Borodovsky M.** 2018. Modeling leaderless transcription and atypical genes results in more accurate gene prediction in prokaryotes. *Genome Res.* **28**, 1079–1089.