Korean Journal of Microbiology (2019) Vol. 55, No. 1, pp. 83-85
DOI https://doi.org/10.7845/kjm.2019.9008

pISSN 0440-2413
eISSN 2383-9902

Draft genome sequence of humic substance-degrading *Pseudomonas* sp. PAMC 29040 from Antarctic tundra soil

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천연 복합유기화합물인 부식질을 분해하는 남극 툰드라 토양 *Pseudomonas* sp. PAMC 29040의 유전체 분석

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(Received January 17, 2019; Accepted March 11, 2019)

Pseudomonas sp. PAMC 29040 was isolated from a maritime tundra soil in Antarctica for its ability to degrade lignin and subsequently confirmed to be able to depolymerize heterogeneous humic substance (HS), a main component of soil organic matter. The draft genome sequences of PAMC 29040 were analyzed to discover the putative genes for depolymerization of polymeric HS (e.g., dye-decolorizing peroxidase) and catabolic degradation of HS-derived small aromatics (e.g., vanillate *O*-demethylase). The information on degradative genes will be used to finally propose the HS degradation pathway(s) of soil bacteria inhabiting cold environments.

Keywords: cold-adapted bacteria, degradative enzyme, soil humic substance

Humic substance (HS) is a naturally occurring complex heteropolymer, which is widely distributed in various environments including Arctic and Antarctic tundra soils. HS is composed mainly of aromatic, aliphatic, and phenolic components, which are covalently bound mostly through various types of C-C and C-O-C bonds (Grinhut *et al*., 2007). Owing to its complexity and structural similarity to lignin, HS has been considered as modified lignin and thus to be rather recalcitrant to microbial degradation. Although the information on HS bacterial degradation, such as catalytic enzymes, is not sufficient, a degradative pathway has been proposed based on several previous fragmentary studies (Bugg *et al*., 2011; Kamimura *et al*., 2017; Kim *et al*., 2018). It is assumed that HS is depolymerized by bacterial extracellular oxidoreductases, such as dye-decolorizing peroxidases and laccases. The resulting HS-derived small aromatic compounds including various biaryls (e.g., β-aryl ether and biphenyl) are generally funneled into a main metabolite, vanillate, which is *O*-demethylated to produce protocatechuate. As another route, vanillate is decarboxylated into guaiacol, which is subsequently *O*-demethylated to catechol (Alvarez-Rodriguez *et al*., 2003). Protocatechuate and catechol are further degraded to TCA cycle intermediates (e.g., acetyl-CoA) through *meta*- or *ortho*-cleavage pathway.

A study site (designated KS1) for HS microbial degradation was selected around Kaya Hill near the Korean Antarctic Research Station (King Sejong) on the Barton Peninsula of King George Island on the western Antarctic Peninsula. In January, 2015, a bacterial strain (PAMC 29040) was isolated

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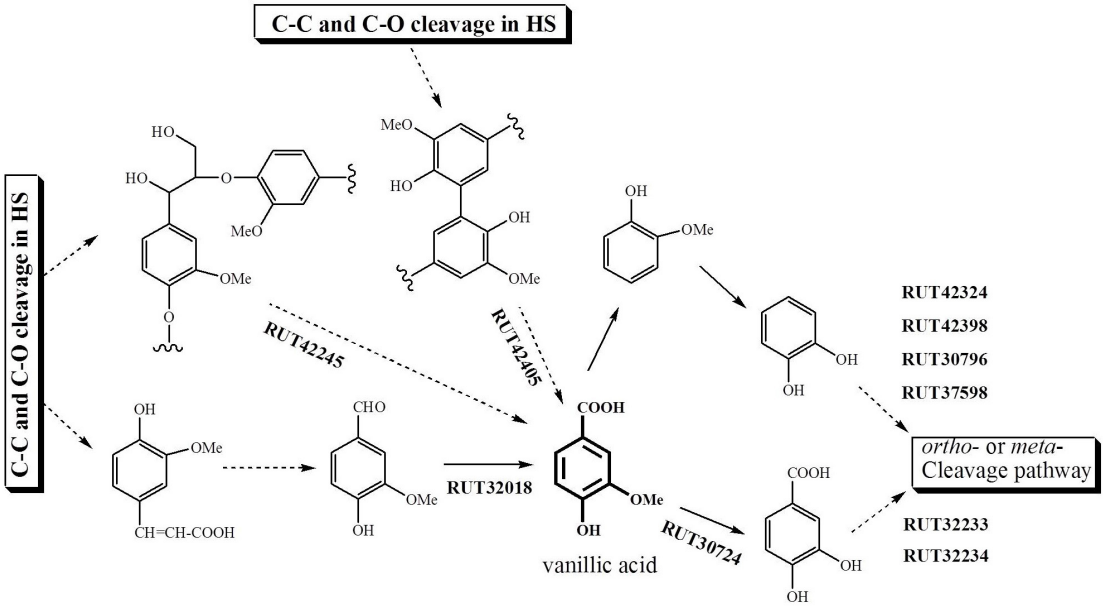

Fig. 1. Proposed HS-degradative pathway by *Pseudomonas* sp. PAMC 29040. Dotted and solid lines represent multi-step reactions by different enzymes and one-step reactions by one enzyme, respectively. GenBank accession numbers for putative enzymes catalyzing the corresponding reactions are shown next the lines.

from a HS-rich tundra soil (KS1-3) owing to its ability to degrade lignin. PAMC 29040 was confirmed to have a degradative capability for heteropolymeric HS and lignin-derived monoaryls (ferulic acid and guaiacol).

The 16S rRNA gene (GenBank no. MK332581) analysis showed that PAMC 29040 was phylogenetically closest to *Pseudomonas weihenstephanensis* DSM 29166^T (99.86% identity) and thus was tentatively named *Pseudomonas* sp. PAMC 29040 (= KCTC 72094). Genome sequencing of PAMC 29040 was performed at ChunLab, Inc. using the Illumina Miseq sequencing method and the sequence was assembled *de novo* into 33 contigs with SPAdes 3.10.1 (Bankevich *et al*., 2012). The draft genome sequence was approximately 5.3 Mb long with a G+C content of 58.6%. The resulting N_{50} size of contigs was 400,925 bp and the total coverage over the genome was 385−fold. Following NCBI GenBank submission, the genes in draft genome sequence were annotated with prokaryotic genome annotation pipeline using best-placed reference protein set; GeneMarkS-2 method (Lomsadze *et al*., 2018). The genome annotation revealed 4,701 coding sequences (CDSs), 60 tRNA genes, and 11 rRNA genes containing one gene for complete 16S rRNA. In the draft genome, several putative HS-degradative genes were detected and used to propose a degradation pathway by PAMC 29040 (Fig. 1): dye-decolorizing peroxidase (GenBank no. RUT42245), biphenyl 2,3-dioxygenase (RUT42405), vanillin dehydrogenase (RUT32018), vanillate *O*-demethylase (RUT 30724), protocatechuate 3,4-dioxygenase for *ortho*-ring cleavage (RUT32233 and RUT32234), catechol 2,3-dioxygenase for *meta*-ring cleavage (RUT42324 and RUT42398), and catechol 1,2-dioxygenase for *ortho*-ring cleavage (RUT30796 and RUT 37598).

Nucleotide sequence accession number

This whole genome shotgun project has been deposited at DBJ/ENA/GenBank under the accession RZAA00000000. The version described in this paper is version RZAA01000000 and consists of sequences RZAA01000001-RZAA01000033.

적 요

남극 연안 툰드라 토양에서 리그닌 분해능이 있는 *Pseudomonas* sp. PAMC 29040를 분리하였으며, 이후 토양 유기물의 주요 구성성분인 복합유기화합물 부식질 분해능을 확인하였다. 부식질 초기 저분자화 효소(예, dye-decolorizing peroxidase)와 부식질 유래의 다양한 저분자 분해산물들을 분해하는 효소들

(예, vanillate *O*-demethylase)를 탐색하기 위해 PAMC 29040 게놈 염기서열을 분석하였다. 분석을 통해서 최종 확보한 효소유전자 정보는 저온환경에 서식하는 토양 세균의 부식질 분해경로 제안에 활용될 것이다.

Acknowledgements

This work was supported by a grant, modeling responses of terrestrial organisms to environmental changes on King George Island (PE19090), funded by the Korea Polar Research Institute.

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