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Complete Genome Sequence of *Massilia* sp. KACC 81254BP Reveals a Potential for Degrading Polyhydroxyalkanoates

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***Massilia* sp. KACC 81254BP, isolated from a landfill on Jeju Island, Republic of Korea, possesses the capability to degrade polyhydroxyalkanoates (PHAs). The genomic analysis of strain KACC 81254BP consists of a circular chromosome comprising 5,028,452 base pairs with a DNA G+C content of 64.6%. This complete genome consists of a total of 4,513 genes, including those encoding the PHA degradation enzyme (*PhaZ*). This study offers valuable genomic insights into the enzymes responsible for degrading polyester plastics.**

Keywords: **Bacteria, *Massilia*, polyhydroxyalkanoate degradation, genome**

Polyhydroxyalkanoates (PHAs) are known to be biodegradable plastics that can replace conventional petrochemical-derived plastics [1], but there is still limited information on the bacteria and their enzymes that degrade PHAs. To date, it is known that manufactured PHA can be degraded by microorganisms, such *as Alkaligenes pecalis, Iliobacter delafieldii*, and *Schlegelella thermodipolymerans*, which can synthesize and degrade PHAs [2]. These degradation is facilitated by the presence of the enzyme PHA depolymerase (*PhaZ*) [3]. In order to obtain bacteria capable of degrading PHA in the laboratory, we screened PHA-degrading microorganisms on plates. As a result, strain KACC 81254BP, which belongs to the genus *Massilia*, was found to be capable of degrading PHA. To identify the degradation genes of this strain, genomic analysis was performed as follows.

Massilia sp. KACC 81254BP was subcultured on R2A agar medium for 2 days at 28℃. The genomic DNA was extracted using the Qiagen MagAttract high-molecular-weight (HMW) DNA kit (Qiagen, Germany) by Macrogen (Republic of Korea), and genome was sequenced by the PacBio Sequel IIe sequencing platform (Pacific Biosciences, USA) and Illumina NovaSeq 6000 (Illumina, USA). For PacBio Sequel sequencing, SMRTbell template was used for the generation of a single-SMRT cell, and the results were assembled de novo using SMRT Link pipeline (version 11.0) [4]. Obtained NovaSeq reads were used to correct error base pair to improve accuracy by Pilon (version 1.21) [5]. Genome annotation was conducted using the NCBI Prokaryotic Genome Annotation Pipeline software (PGAP; version 6.5) and Prokka (version 1.14.6). Total 108,193 subreads were generated in PacBio Sequel IIe and 1,718,966,607 qualified filtered reads were generated in illumina NovaSeq 6000. The genome of *Massilia* sp. KACC 81254BP was generated as a single circular chromosome (5,028,452 bp, 64.6% G+C content, 322.6x genome coverage), with no plasmid. The genome consists of 4,513 protein-coding genes (CDS), including 19 rRNAs, 69 tRNAs and 4 ncRNAs annotated by both of PGAP. Annotated genes were also categorized using the egg-

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NOG-mapper (version 2) [6]. Based on the eggNOG annotation results, protein coding genes are mainly involved in signal transduction mechanisms (340 genes), amino acid transport and metabolism (326 genes), cell wall/membrane/envelope biogenesis (299 genes), and transcription (260 genes), while 779 genes are categorized as unknown functions.

For the identification of strain at the species level, the orthologous average nucleotide identity (OrthoANI) value between *Massilia* sp. KACC 81254BP and *M. frigida* CCM 8695^{T} (WHJG01000115), which is the closet species based on the 16S rRNA gene similarity, was calculated [7]. The OrthoANI value (80.0%) indicates that *Massilia* sp. KACC 81254BP could potentially be distinguished as a novel species within the genus *Massilia* [8].

The amino acid sequences and reported PHA-related enzyme sequences [9] were aligned and compared by sword (version 1.0.4). In the comparison, genes analyzed to decompose plastic were searched based on identification of more than 50%. The PHA-related cluster genes obtained from Prokka were visualized on the genome map by ggplot2 (version 3.4.3) in R (version 4.1.3) (Fig. 1). We identified six genes related to the PHA-related cluster including *PhaR* (Q4S45_07655), *PhaA* (Q4S45_19985), *PhaZ* (Q4S45_02195), *PhaB* (Q4S45_19980), *PhaC* (Q4S45_07650), *PhaB* (Q4S45_04890) by comparing genome of *C. necator* H16 (Table 1). Among them, *PhaZ* encodes a PHA-depolymerase, which is well-known for their ability to break down polyhydroxyalkanoates (PHAs) [10]. Based on these results, we expected that the PHA degradation ability of *Massilia* sp. KACC 81254BP comes from the expression of the genes of PHA depolymerase. We confirmed the PHA degrading related genes of *Massilia* sp. KACC 81254BP based on genome sequencing. These results will help to analyze the gene expression involving the PHA degradation process.

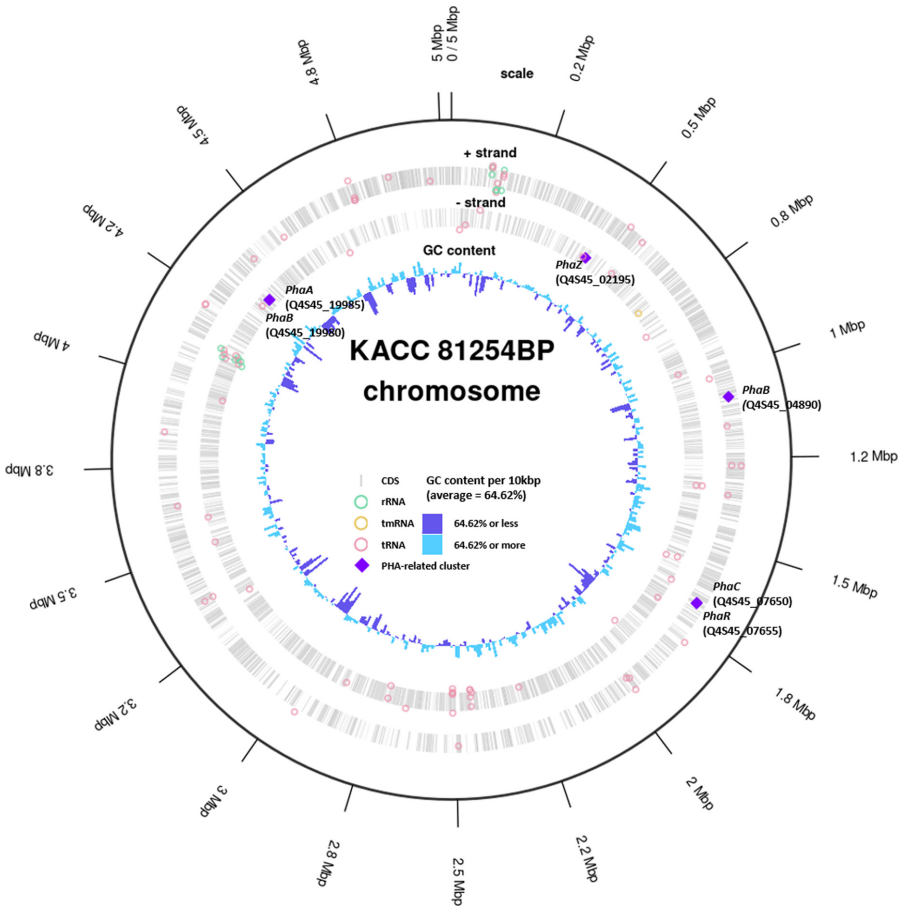

Fig. 1. Circular map of the *Massilia* sp. KACC 81254BP chromosome. The symbol (◆) indicates PHA relating genes.

Table 1. PHA-related cluster characteristics of the *Massilia* sp. KACC 81254BP.

PHA-related cluster*	Protein (name)	Locus tag	Similarity (%)
PhaA	acetyl-CoA C-acetyltransferase	Q4S45_19985	74
PhaB	acetoacetyl-CoA reductase	Q4S45_19980	61
PhaB	acetoacetyl-CoA reductase	Q4S45_04890	52
PhaC	class I poly(R)-hydroxyalkanoic acid synthase	Q4S45_07650	60
PhaM	-	-	-
PhaP	-	-	-
PhaR	polyhydroxyalkanoate synthesis repressor *PhaR*	Q4S45_07655	75
PhaZ	Polyhydroxyalkanoate depolymerase	Q4S45_02195	65

*PHA-related gene and protein sequences of the model organism *Cupriavidus necator* H16 (AM260479) [9].

The complete genome sequence of *Massilia* sp. KACC 81254BP (CP131935) has been deposited in the NCBI database.

요 약

제주도 매립지에서 분리된 *Massilia* strain KACC 81254BP는 생분해성플라스틱 폴리하이드록시알카노에이트(PHA)를 분해할 수 있다. 이 균주의 유전체는 5,028,452 bp의 원형 염색체로 구성되어 있으며, G+C 함량은 64.6%이다. 이 유전체는 PHB depolymerase를 포함한 4,513개 유전자가 확인되었다. 이 유전체는 신호전달과 아미노산 수송 등 대사와 관련한 다양한 유전자를 포함하고 있다. 이 연구는 *Massilia* sp. KACC 81254BP의 폴리에스터 플라스틱 분해 효소와 관련된 유전학적 정보를 제공한다.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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