# Dechloromonas hankyongensis sp. nov., isolated from wetland<sup>§</sup>

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# 습지로부터 분리된 신종 Dechloromonas hankyongensis<sup>§</sup>

김지원<sup>1</sup> · 조정훈<sup>1,2</sup> · 임완택<sup>1,2,3\*</sup>(D)

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A Gram-stain-negative, rod-shaped, motile via polar flagellum, facultatively anaerobic, creamy, bacterium (designated XY25<sup>T</sup>) was isolated from wetland from Eco Park in Godeok-dong, Gangdong-gu, Seoul, Republic of Korea. On the basis of 16S rRNA gene sequencing, strain XY25<sup>T</sup> clustered with species of the Dechloromonas and appeared closely related to Dechloromonas agitata CKB<sup>T</sup> 98.49%. Dechloromonas hortensis MA-1<sup>T</sup> 98.15%. and Dechloromonas denitrificans ATCC BAA-841<sup>T</sup> 97.67%. The average nucleotide identity (ANI) calculated between strain XY25<sup>T</sup> and each of the three strains (*Dechloromonas agitata* CKB<sup>T</sup>, Dechloromonas hortensis MA-1<sup>T</sup>, and Dechloromonas denitrificans ATCC BAA-841<sup>T</sup>) were 83.7, 81.2, and 81.0 %. And the digital DNA-DNA hybridization (dDDH) calculated between strain XY25<sup>T</sup> and each of the three strains (Dechloromonas agitata CKB<sup>T</sup>, Dechloromonas hortensis MA-1<sup>T</sup>, and Dechloromonas denitrificans ATCC BAA-841<sup>T</sup>) were 26.5, 23.5, and 23.8 %. Growth occurs at 18-37°C on R2A medium in the presence of 0-0.5% NaCl (w/v) and at pH 5.5-8.0. The DNA G + C content of the genomic DNA was 62.9 mol%, and ubiquinone-8 (Q-8) was the major respiratory quinone. The major cellular fatty acids (> 5%) were of summed feature 3 ( $C_{16:1} \omega 7c/C_{16:1} \omega 6c$ ),  $C_{16:0}$ , C12:0, C10:0 3OH, summed feature 2 (C14:0 3OH/C16:1 iso I), and summed feature 8 ( $C_{18:1} \omega 7c/C_{18:1} \omega 6c$ ). The polar lipids consisted of phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and diphosphatidylglycerol (DPG). Physiological and

biochemical characteristics indicated that strain  $XY25^{T}$  represents a novel species of the genus *Dechloromonas*, for which the name *Dechloromonas hankyongensis* sp. nov. is proposed. The type strain is  $XY25^{T}$ (= KACC 22221<sup>T</sup> = LMG 32191<sup>T</sup>).

Keywords: Dechloromonas, polyphasic taxonomy, wetland, 16S rRNA gene sequence

Wetland soils are excellent habitats for a variety of microorganisms such as bacteria, fungi and protozoa, and their lack of oxygen content makes them a beneficial habitat for all kinds of bacterial communities aerobic, anaerobic and facultative (Mohan and Tippa, 2019). Also soil holds many contaminants, during which it releases back to surface water and engages in all biogeochemical processes catalyzed by microorganisms (Mohan and Tippa, 2019). Natural processes, including biogeochemical processes, can't be achieved or understood without the assistance of microorganisms (Mohan and Tippa, 2019). Thus, the role of microbes plays an important role in understanding all natural processes (Mohan and Tippa, 2019). These microorganisms perform a variety of functions, from mineralization processes to bioavailability (Mohan and Tippa, 2019).

The *Dechloromonas* resides with the family *Azonexaceae*, which belongs to the class *Betaproteobacteria* (Achenbach *et al.*, 2001). Members of the *Dechloromonas* commonly isolated from wetland environments, usually from pristine and conta-

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minated soils (Achenbach *et al.*, 2001), sediments and waste sludges (Achenbach *et al.*, 2001), the gut of the earthworm Aporrectodea caliginosa (Horn *et al.*, 2005), polluted and pristine sites (Wolterink *et al.*, 2005). All of them were generally Gram-negative, mesophilic, motile by a single polar flagellum, facultatively anaerobic, non spore-forming, non-fermentative rods (Achenbach *et al.*, 2001). Currently 8 *Dechloromonas* species have been described (http://www.bacterio.net/). In this study, we describe the isolation and identification of a novel species of the genus *Dechloromonas*.

#### Materials and Methods

#### Strain isolation

To screen for bacterial strains living in soil from wetland in Eco Park, Godeok-dong, Gangdong-gu, Seoul, Republic of Korea (37°34'02.2"N 127°08'59.1"E). Wetland soil samples were collected from wetland environments that can be easily found in Republic of Korea. The samples were carefully suspended in R2A broth and carefully shaking R2A broth containing wetland samples, the slurry was diluted from 10<sup>-1</sup> to 10<sup>-4</sup>, spread on R2A (Difco) plates. Then, the plates were incubated at 30°C for 1 week. After 1 week, the strains were purified by subculturing on new R2A plates. We secured a total of 72 colonies, of which 12 novel species candidate strains were excavated. At this time, the identified strains were genera Solibacillus, Acinetobacter (2 strains), Ramlibacter, Dechloromonas, Ramlibacter, Sphingomonas, Pelomonas, Arthrobacter, Terrimonas, Massilia, and Undibacterium. They were selected mainly for well-growing strains. Among them *Dechloromonas* sp. XY25<sup>T</sup> was routinely cultured on R2A agar and maintained in a glycerol suspension (R2A broth with 20 %, v/v), at -80°C.

#### Information on reference strains

In this current report, we describe a novel bacterial strain, designated XY25<sup>T</sup>, which appears to be a member of the genus *Dechloromonas*. Reference strains (*Dechloromonas agitata* CKB<sup>T</sup>, *Dechloromonas hortensis* MA-1<sup>T</sup>, *Dechloromonas denitrificans* ATCC BAA-841<sup>T</sup>) were obtained respectively from German Collection of Microorganisms and Cell Cultures

(DSMZ) and Japan Collection of Microorganisms (JCM) for use in a comparative analysis.

### Morphological, physiological and biochemical characterization

The Gram staining was determined using the described method of Buck (1982). Cell shape, size, and the presence of flagella were determined under a LIBRA 120 (120 kV) transmission electron microscope (Carl Zeiss) and Nikon light microscopy (×1000 magnification), after cells grown for 2 days at 30°C on R2A medium. Motility was checked on R2A broth supplemented with 0.2% agar (Weon et al., 2008). Cell growth of strain XY25<sup>T</sup> was monitored at various temperatures 4, 10, 18, 25, 30, 37, 42, 45, and 50°C, respectively. Various initial pH values (5.5-8.0 at intervals of 0.5 pH units) evaluated after 7 days of incubation at 30°C using R2A broth. The following buffers (each 20 mM final concentration) were used to adjust the pH of R2A broth: acetate buffer for pH 4.0-5.5, phosphate buffer for pH 6.0-8.0 and Tris buffer for pH 8.5-10.0. Salt tolerance was tested in a R2A broth that controlled only the concentration of sodium chloride in the composition of the R2A medium supplemented with 0.5% and 1 to 10% (w/v at intervals of 1% unit) NaCl and growth assessed after 7 days of incubation at 30°C. An anaerobic growth test was conducted with the GasPak<sup>TM</sup> EZ anaerobe pouch system (BD) over two weeks. Tests for the hydrolysis of Tween-60, casein, starch, carboxyl methyl cellulose (Cowan and Steel, 1974; Atlas, 1993), and DNA (using DNase agar from Scharlau, with DNase activity detected by flooding plates with 1 M HCl) were carried out after 5 days of incubation at 30°C. Biochemical tests were carried out using commercial API (API 20NE, API ID 32GN, and API ZYM) kits according to the manufacturer (bioMérieux) instructions. The API ZYM test strip was read after 4 h of incubation at 37°C, and the other API strips were examined after 2 days at 30°C. Catalase and oxidase activities were determined as previously described (Cappuccino and Sherman, 2002).

#### Phylogenetic analysis

Genomic DNA of strain XY25<sup>T</sup> was isolated using a genomic DNA extraction kit (Macrogen Co. Ltd) for 16S rRNA sequence

and genome sequence, and the 16S rRNA gene was amplified using the universal bacterial primer set (907R and 785F) (Lane, 1991). Then, the purified PCR products were sequenced by Macrogen Co. Ltd. The sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR) and the 16S rRNA gene sequences of related taxa, which were obtained from the GenBank database and EzTaxon-e server (http://www. ezbiocloud.net) (Yoon et al., 2017a). Multiple alignments were performed by Clustal X program with gaps edited in BioEdit program (Thompson et al., 1997; Hall, 1999). Neighbor-joining (NJ), Maximum-likelihood (ML), and maximum-parsimony (MP) trees were constructed using the Molecular Evolutionary Genetics Analysis 7 (MEGA 7.0) software with bootstrap analysis based on 1,000 replications. Kimura two parameter model was used for the ML and NJ tree construction with complete deletion of gapes While MP tree was made with Subtree-Pruning-Regrafting heuristic method with gaps of complete deletion (Fitch, 1971; Kimura, 1980; Felsenstein, 1985; Saitou and Nei, 1987; Kumar et al., 2016).

#### Draft genome sequencing and G + C content analysis

The minimal standards for use of genome data in taxonomy of prokaryotes leaded these analyses (Chun et al., 2018). The draft genomic sequencing of strain XY25<sup>T</sup> was performed by Illumina NovaSeq analysis and assembled using the SPAdes v0.4.7 de novo assembler. The draft genome sequence was submitted to the GenBank database (www.ncbi.nlm.nih.gov) and annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) (Tatusova et al., 2016). In the case of genome annotaion, it was further analyzed based on RAST (Aziz et al., 2008). Compare as a pair each genome-based relatedness between strain XY25<sup>T</sup> and closely related strain, *Dechloromonas* agitata CKB<sup>T</sup>, Dechloromonas hortensis MA-1<sup>T</sup>, and Dechloromonas denitrificans ATCC BAA-841<sup>T</sup> were in pairs with estimated based on the average nucleotide identity (ANI) using the ANI calculator employing the OrthoANIu algorithm (Yoon et al., 2017b) available from the EzBioCloud service. The digital DNA-DNA hybridization (dDDH) value was calculated using the online Genome to Genome Distance Calculator (http:// ggdc.dsmz.de/ggdc.php) (Li et al., 2019). Furthermore, the phylogenetic tree based on the whole-genome sequencing using automated multi-locus species tree analysis (Alanjary et *al.*, 2019) and Neighbor-joining (NJ) on Molecular Evolutionary Genetics Analysis 7 (MEGA 7.0) software with bootstrap analysis based on 1,000 replications, Kimura two parameter model (Fitch, 1971; Kimura, 1980; Felsenstein, 1985; Saitou and Nei, 1987; Kumar *et al.*, 2016). And, G + C content was calculated from the genome sequence analysis results.

#### Chemotaxonomic analysis

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions, and reextracted in n-hexane/water (1:1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac cartridges silica (Waters) and subsequently analyzed by HPLC as previously described (Hiraishi *et al.*, 1996). Cellular fatty acids profiles were determined for strains grown on R2A medium for 2 days at 30°C. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analysed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). Strain XY25<sup>T</sup> was examined for their polar lipid contents as described previously (Minnikin *et al.*, 1984).

#### Results

#### Physiological characteristics

Colonies of strain XY25<sup>T</sup> grown on R2A agar plates for 2 days at 30°C were round, entire, flat, cream colored (Supplementary data Fig. S1). Cells were Gram-stain-negative, facultatively aerobic, spore-forming, motile by a single polar flagellum, and rod-shaped (0.2–0.9  $\mu$ m in diameter and 1.2–2.5  $\mu$ m in length) (Supplementary data Fig. S2). Positive for Cellulose but not casein, Tween-60, starch and DNase. Growth occurs at 18– 37°C (optimum, 30°C), at pH 5.5–8.0 (optimum, pH 6.5) and in the presence of 0–0.5% (w/v) NaCl (optimum, 0 %). And For all comparative strains, optimal growth was confirmed at 30– 36°C in temperature, 7–7.5 in pH, and 0% in NaCl. More inform of the physiological and biochemical characteristics of strain XY25<sup>T</sup> are summarized in the description and Table 1.

#### Table 1. Physiological and biochemical characteristics between strain XY25<sup>T</sup> and closely related species of the genus *Dechloromonas*

1, Dechloromonas hankyongensis XY25<sup>T</sup>; 2, Dechloromonas agitata CKBT; 3, Dechloromonas hortensis MA-1<sup>T</sup>; 4, Dechloromonas denitrificans ATCC BAA-841<sup>T</sup>.

All tests were obtained in this study. All strains are positive for 3-hydroxy-butyrate, esterase (C4), esterase lipase (C8), naphtol-AS-BI-phosphohydrolase. Negative for indole production, glucose acidification, arginine dihydrolase,  $\beta$ -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis),  $\beta$ -galactosidase (PNPG), L-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, D-maltose, gluconate, caprate, adipate, citrate, salicin, D-melibiose, L-fucose, D-sorbitol, L-histidine, 2-ketogluconate, 4-hydroxy-benzoate, L-proline, L-rhannose, D-ribose, inositol, D-sucrose, itaconate, suberate, malonate, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, L-serine, lipase (C14), valine arylamidase, cystine arylamidase, trypsin,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -glucosidase,  $\beta$ -glucosidase,  $\beta$ -glucosidase,  $\beta$ -glucosidase,  $\alpha$ -flucosidase,  $\alpha$ -glucosidase,  $\beta$ -glucos

| Characteristics                       | 1       | 2  | 3  | 4   |
|---------------------------------------|---------|--|--|---|
| Isolation source                      | Wetland | Sediments and waste sludges <sup>a</sup> | Polluted and pristine sites <sup>b</sup> | Earthworm<br>Aporrectodea caliginosa <sup>c</sup> |
| API 20 NE & ID 32 GN tests            |         |  |  |   |
| Nitrate reduction $(NO_3^- > NO_2^-)$ | +       | -  | -  | -   |
| Urease                                | -       | -  | +  | -   |
| D-Glucose                             | +       | -  | -  | -   |
| Malate                                | +       | -  | +  | -   |
| Phenyl-acetate                        | +       | -  | -  | +   |
| Propionate                            | +       | -  | +  | -   |
| Valerate                              | +       | -  | -  | W   |
| Acetate                               | +       | -  | W  | -   |
| Lactate                               | +       | +  | +  | W   |
| L-Alanine                             | -       | -  | -  | +   |
| API ZYM tests                         |         |  |  |   |
| Alkaline phosphatase                  | -       | -  | +  | +   |
| Leucine arylamidase                   | +       | W  | +  | +   |
| Acid phosphatase                      | +       | -  | -  | +   |
| G + C content (mol%)                  | 62.9    | 62.9                                     | 61.4                                     | 61.7  |

\*Data from; <sup>a</sup>Achenbach et al. (2001); <sup>b</sup>Wolterink et al. (2005); <sup>c</sup>Horn et al. (2005).

<sup>†</sup>The DNA G + C content of strain XY25<sup>T</sup> was calculated from its genome.

#### Phylogenetic tree analysis

The complete 16S rRNA gene sequence (1,460 bp) of strain XY25<sup>T</sup> was determined and subjected to a comparative analysis. The novel isolate was found to belong to the *Dechloromonas* (Fig. 1, Supplementary data Figs. S3 and S4) and indicated highest sequence similarity to *Dechloromonas agitata* CKB<sup>T</sup> (AF047462) (98.49%), *Dechloromonas hortensis* MA-1<sup>T</sup> (AY277621) (98.15%), and *Dechloromonas denitrificans* ATCC BAA-841<sup>T</sup> (LODL01000012) (97.67%). Based on 16S rRNA gene sequence and phylogenetic tree analyses, these strains were used as reference strains in most of the phenotypic analyses. And bootstrap value of 1,000 replications analyses showed that *Dechloromonas hankyongensis* XY25<sup>T</sup> was tied to *Dechloromonas agitata* CKB<sup>T</sup> at 98%, *Dechloromonas hortensis* MA-1<sup>T</sup> was tied to *Dechloromonas denitrificans* ATCC BAA-

841<sup>T</sup> at 100%, and *Dechloromonas hankyongensis* XY25<sup>T</sup>, *Dechloromonas agitata* CKB<sup>T</sup>, *Dechloromonas hortensis* MA-1<sup>T</sup>, and *Dechloromonas denitrificans* ATCC BAA-841<sup>T</sup> strains were grouped to 62%.

#### Draft genome sequencing and G + C content analysis

The genome of strain XY25<sup>T</sup> consists of a chromosome with 4,095,330 bp and a G + C content of 62.9 mol%, consisting of 16 contigs with an N50 value of 1,479,842 bp. The average sequencing depth of coverage was determined to be 1813.8X. The 16S rRNA gene sequence made using Sanger sequencing methods was 100% identical to those gene extracted from the XY25<sup>T</sup> annotated genome. The genome includes 3,861 coding genes (CDSs), 3 rRNAs, 55 tRNAs, and 4 ncRNAs. According to the genome annotation based on RAST (Aziz *et al.*, 2008), a



Fig. 1. Neighbor-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of XY25<sup>T</sup> with other related species of the genus *Dechloromonas*. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithm. Bootstrap values expressed as percentages of 1,000 replications greater than 60% are shown at the branch points. Bar, 0.01 substitutions per nucleotide position.

number of genes related to nitrogen metabolism and denitrification were encoded in the genome of the XY25<sup>T</sup> (Supplementary data Fig. S5). In addition, considering that it has a Nar (NarR, NarK, NarG, NarH, NarJ, and NarI) gene cluster, Nor (NorD, NorQ, NorB, NorC, NorF, and NorE) gene cluster, and Nos (NosX, NosL, NosY, NosF, NosD, NosZ, and NosR) gene cluster, it is presumed that it has a denitrification ability following the nitrate  $\rightarrow$ nitrite  $\rightarrow$ nitric oxide  $\rightarrow$ nitrous oxide  $\rightarrow$  dinitrogen process.

The average nucleotide identity (ANI) calculated between strain XY25<sup>T</sup> and each of the three strains (*Dechloromonas gitate* CKB<sup>T</sup>, *Dechloromonas hortensis* MA-1<sup>T</sup>, *Dechloromonas denitrificans* ATCC BAA-841<sup>T</sup>) were 83.7, 81.2, and 81.0 %, respectively. And the digital DNA-DNA hybridization (Dddh) calculated between strain XY25<sup>T</sup> and each of the three strains (*Dechloromonas gitate* CKB<sup>T</sup>, *Dechloromonas hortensis* MA-1<sup>T</sup>,

*Dechloromonas denitrificans* ATCC BAA-841<sup>T</sup>) were 26.5, 23.5, and 23.8%, respectively, and which were below the proposed ANI cut-off values of 95–96% and Dddh cut-off values of 70% for interspecies identity (Kim *et al.*, 2014). Genome sequence-based phylogenetic tree analysis results obtained a combination of *Dechloromonas* specialties like 16S Rrna-based phylogenetic tree (Supplementary data Fig. S6).

#### Chemotaxonomic analysis

The major quinone detected in strain XY25<sup>T</sup> was ubiquinone-8 (Q-8), which is same to other species in genus *Dechloromonas*. The major cellular fatty acids of strain XY25<sup>T</sup> were mainly composed of summed feature 3 (C<sub>16:1</sub>  $\omega$ 7*c*/C<sub>16:1</sub>  $\omega$ 6*c*), C<sub>16:0</sub>, C<sub>12:0</sub>, C<sub>10:0</sub> 3OH, summed feature 2 (C<sub>14:0</sub> 3OH/C<sub>16:1</sub> iso I), and summed feature 8 (C<sub>18:1</sub>  $\omega$ 7*c*/C<sub>18:1</sub>  $\omega$ 6*c*) which is similar to those of described species in the *Dechloromonas* (Table 2). The

# Table 2. Fatty acid profiles of strain XY25<sup>T</sup> and related species of the genus *Dechloromonas*

1, Dechloromonas hankyongensis XY25<sup>T</sup>; 2, Dechloromonas agitata CKBT; 3, Dechloromonas hortensis MA-1<sup>T</sup>; 4, Dechloromonas denitrificans ATCC BAA-841<sup>T</sup>.

All strains were cultured on R2A medium for 48 h at 30°C. Some fatty acids amounting to < 0.5% of the total fatty acids in all strains are not listed. tr, trace amounting (< 0.5%); –, not detected.

| Fatty acid   | 1    | 2    | 3    | 4    |
|--|------|------|------|------|
| Saturated  |      |      |      |      |
| C <sub>12:0</sub>  | 8.1  | 7.9  | 0.8  | 2.5  |
| C14:0  | 1.7  | 2.1  | 3.4  | 1.1  |
| C <sub>16:0</sub>  | 17.4 | 25.5 | 20.2 | 21.3 |
| Unsaturated  |      |      |      |      |
| $C_{16:1} \omega 5c$   | 0.3  | tr   | 1.2  | 1.1  |
| Hydroxy fatty acids  |      |      |      |      |
| C <sub>10:0</sub> 3OH  | 4.7  | 4.6  | 3.4  | 3.4  |
| C <sub>12:1</sub> 3OH  | tr   | tr   | 2.9  | 2.7  |
| Cyclo  |      |      |      |      |
| C <sub>17:0</sub> cyclo  | tr   | tr   | 1.8  | tr   |
| Summed feature   |      |      |      |      |
| 2; C <sub>14:0</sub> 3OH/C <sub>16:1</sub> iso I                       | 2.6  | 2.0  | tr   | tr   |
| <b>3;</b> C <sub>16:1</sub> ω7 <i>c</i> /C <sub>16:1</sub> ω6 <i>c</i> | 60.8 | 40.5 | 53.1 | 58.4 |
| <b>7;</b> C <sub>19:1</sub> ω6c/.846/19cy                              | tr   | tr   | 0.5  | 0.7  |
| <b>8;</b> C <sub>18:1</sub> ω7 <i>c</i> /C <sub>18:1</sub> ω6 <i>c</i> | 2.4  | 17.5 | 12.6 | 8.3  |

\*Summed Features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total.

major polar lipids were phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and diphosphatidylglycerol (DPG) (Supplementary data Fig. S7). From the polar lipid analysis, the novel isolate was found to share major polar lipids PG, PE, and DPG with described species in the genus *Dechloromonas*.

#### Discussion

Based on our taxonomic and morphological analyses, strain  $XY25^{T}$  shares major Q-8 as ubiquinone, and summed feature 3 (C<sub>16:1</sub>  $\omega$ 7*c*/C<sub>16:1</sub>  $\omega$ 6*c*), C<sub>16:0</sub>, C<sub>12:0</sub>, C<sub>10:0</sub> 3OH, summed feature 2 (C<sub>14:0</sub> 3OH/C<sub>16:1</sub> iso I), and summed feature 8 (C<sub>18:1</sub>  $\omega$ 7*c*/C<sub>18:1</sub>  $\omega$ 6*c*) as major fatty acids (CFAs) and phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and diphosphatidylglycerol (DPG) as major polar lipids with described species in the genus *Dechloromonas*. However, even though the phylogenetic tree based on 16S rRNA gene sequences places XY25<sup>T</sup> in the same group as *Dechloromonas agitata* CKB<sup>T</sup> (98.49%), *Dechloromonas* 

*hortensis* MA-1<sup>T</sup> (98.15%), and *Dechloromonas denitrificans* ATCC BAA-841<sup>T</sup> (97.67%), chemotaxonomic and phenotypic characteristics differentiate the novel isolate from this the latter *Dechloromonas* species (Tables 1 and 2). Therefore, strain XY25<sup>T</sup> represents a novel species in the *Dechloromonas* for which the name *Dechloromonas hankyongensis* sp. nov. is proposed.

#### Description of Dechloromonas hankyongensis sp. nov.

Dechloromonas hankyongensis (han.kyong.en'sis. N.L. masc./ fem. adj. hankyongensis, pertaining to Hankyong National University Republic of Korea, where taxonomic studies of this taxon were performed).

Cells are Gram-stained-negative, facultatively anaerobic, catalase positive but not oxidase. Colonies grown on R2A are round, entire, flat, 0.8 um in diameter and cream colored. Growth occurs at 18-37°C (optimum 30°C) in the presence of 0-0.5% NaCl (optimum 0%) and at pH 5.5-8.0 (optimum pH 6.5) and Colonies grown not on TSA, LB agar, marine agar. Positive for the hydrolysis of CM-cellulose but not casein, DNA, starch and Tween-60. The XY25<sup>T</sup> are positive for nitrate reduction  $(NO_3^- > NO_2^-)$ , D-glucose, malate, phenyl-acetate, propionate, valerate, 3-hydroxy-butyrate, acetate, lactate and negative for indole production, glucose acidification, arginine dihydrolase, urease, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β-galactosidase (PNPG), L-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, D-maltose, gluconate, caprate, adipate, citrate, salicin, D-melibiose, L-fucose, D-sorbitol, L-histidine, 2-ketogluconate, 4-hydroxy-benzoate, L-proline, L-rhamnose, D-ribose, inositol, D-sucrose, itaconate, suberate, malonate, L-alanine, 5-ketogluconate, glycogen, 3hydroxy-benzoate, L-serine in API 20NE & 32GN. Also, XY25<sup>T</sup> are positive for esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase and negative for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin,  $\alpha$ -chymotrypsin,  $\alpha$ galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase,  $\beta$ -glucosidase, N-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase,  $\alpha$ -fucosidase in API ZYM. The predominant guinone is Q-8. The major cellular fatty acids are summed feature 3 ( $C_{16:1} \omega 7c/C_{16:1}$ ω6c), C<sub>16:0</sub>, C<sub>12:0</sub>, C<sub>10:0</sub> 3OH, summed feature 2 (C<sub>14:0</sub> 3OH/C<sub>16:1</sub> iso I), and summed feature 8 ( $C_{18:1} \omega 7c/C_{18:1} \omega 6c$ ). The polar

lipids are phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and diphosphatidylglycerol (DPG). The DNA G + C content of genomic DNA is 62.9 mol%.

The type strain,  $XY25^{T}$  (= KACC 22221<sup>T</sup> = LMG 32191<sup>T</sup>) was isolated from wetland from Eco Park in Godeok-dong, Gangdong-gu, Seoul, Republic of Korea.

The draft genome and 16S rRNA gene sequence of strain XY25<sup>T</sup> has been deposited at GenBank/EMBL/DDBJ under accession numbers JAKLTN000000000 and MW164936, respectively.

## 적 요

서울특별시 강동구 고덕동 생태공원 습지에서 그람 음성으 로 막대 모양이며 운동성을 갖고, 극성편모이며, 선택적 호기성 이면서, 크림색을 띠는 세균(XY25<sup>T</sup>)이 분리되었다. 16SrRNA 유전자 염기서열 분석 결과, XY25<sup>T</sup> 균주는 Dechloromonas agitata CKB<sup>T</sup> (98.49% 염기서열 상동성), Dechloromonas hortensis MA-1<sup>T</sup> (98.15%), Dechloromonas denitrificans ATCC BAA-841<sup>T</sup> (97.67%)과 유사성이 매우 높은 것으로 나타났다.  $\overline{\mathcal{A}}$ 주 XY25<sup>T</sup>와 3개의  $\overline{\mathcal{A}}$ 주(Dechloromonas agitata CKB<sup>T</sup>, Dechloromonas shortensis MA-1<sup>T</sup>, Dechloromonas denitrifitans ATCC BAA-841<sup>T</sup>) 사이에서 계산된 평균 뉴클레오티드 동일 성(ANI)은 83.7, 81.2, 81.0%였다. 그리고 균주 XY25<sup>T</sup>와 3개 의 균주 간에 계산된 디지털 DNA-DNA 혼성화(DDH)값은 26.5, 23.5, 23.8%였다. 균주는 R2A 배지상에서 0-0.5% NaCl (w/v), 18-37°C, pH 5.5-8.0 범위에서 생장이 가능하였다. 유 전체 DNA의 DNA G+C 함량은 62.9 mol%이며, 주요 호흡 퀴 논은 Ubiquinone-8 (Q-8)이었다. 주요 세포 지방산(> 5%)은 summed feature 3 ( $C_{16:1} \omega 7c/C_{16:1} \omega 6c$ ),  $C_{16:0}$ ,  $C_{12:0}$ ,  $C_{10:0}$  3OH, summed feature 2 (C14:0 3OH/C16:1 iso I), summed feature 8 (C<sub>18:1</sub> ω7c/C<sub>18:1</sub> ω6c)이었다. 극성 지질은 포스파티딜글리세 롤(PG), 포스파티딜에탄올아민(PE), 디포스파티딜글리세롤 (DPG)로 구성되었다. 생리학적, 생화학적 특성 분석에 따르 면 XY25<sup>T</sup> 균주가 Dechloromonas 속의 신종임을 나타내며 신 규 학명 Dechloromonas hankvongensis sp. nov.를 제안한다. 표준 균주는 XY25<sup>T</sup>(= KACC 22221<sup>T</sup> = LMG 32191<sup>T</sup>)이다.

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## Conflict of Interest

The authors have no conflict of interest to report.

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