Draft genome sequences of fluoroquinolone resistant Enterobacter hormaechei strains isolated from dental biofilm

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치면 세균막에서 분리된 플루오로퀴놀론 내성 *Enterobacter hormaechei* 균주의 유전체 역기서열

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Enterobacter hormaechei is a nosocomial pathogen isolated mainly from skin wound, sputum, urine, stool, peritoneal fluid, ear discharge, and blood. Two strains of *E. hormaechei* exhibiting resistance to fluoroquinolone, were isolated from dental biofilm of Korean periodontitis patients, and their genomic sequences were analyzed. The genome sizes of KHUD_001 and KHUD_002 were 4,836,070 bp (G + C content: 55.36%) and 4,848,366 bp (G + C content: 55.33%), respectively. Both genomes were found to contain genes encoding multidrug efflux pump systems such as the major facilitator superfamily (MFS) and resistance-nodulation-cell division (RND) that are associated with resistance to antimicrobial agents.

Keywords: *Enterobacter hormaechei*, dental biofilm, fluoroquinolone resistance, genome

The oral cavity has more than 700 species of bacteria, making it the second largest microbiota in the humans after the gut (Deo and Deshmukh, 2019). Of these, only a tiny fraction is associated with oral infections such as dental caries and periodontitis (Zaatout, 2021). Meanwhile, the low but consistent presence of non-oral *Enterobacteriaceae* among the oral

microbiota has been reported (Costa et al., 2021; Zaatout, 2021; Jepsen et al., 2022). Although their exact role in the oral cavity remains to be elucidated, it has been considered that they can contribute to generalized inflammation and disease progression (Costa et al., 2021) and act as potential reservoirs for the development and spread of antibiotic resistance (Jepsen et al., 2022). Enterobacter is a genus belonging to the family of Enterobacteriaceae that is associated primarily with healthcarerelated infections. The Enterobacter cloacae complex contains 7 species with more than 60% DNA-DNA homology (Davin-Regli et al., 2019). Among them Enterobacter hormaechei is one of the most common nosocomial pathogens and mainly isolated from skin wound, sputum, urine, stool, peritoneal fluid, ear discharge, and blood (O'Hara et al., 1989; Wenger et al., 1997; Kremer and Hoffmann, 2012). Here, we report characteristics and genome sequences of two strains of E. hormaechei isolated from dental biofilm of Korean periodontitis patients. These isolates were obtained from Kyung Hee University Dental Hospital (KHD IRB 1606-5). As shown in Fig. 1, Escherichia coli ATCC 25922, a fluoroquinolonesusceptible strain (Li et al., 2019), was completely killed by 0.05 µg/ml ciprofloxacin and 1 µg/ml levofloxacin. By contrast, E. hormaechei strains exhibited resistance to fluoroquinolone

and were completely killed when exposed to $4 \mu g/ml$ of the drugs.

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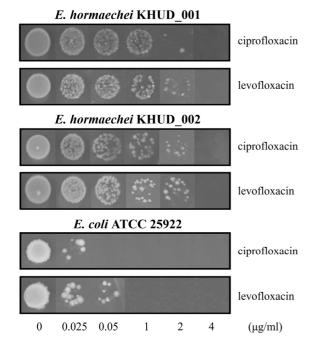


Fig. 1. Growth of *E. hormaechei* KHUD_001 and KHUD_002 in the presence of fluoroquinolones. *Escherichia coli* ATCC 25922 was used as a control strain. Each bacterial strain was grown in Mueller-Hinton broth (Difco Laboratories) with fluoroquinolones at the indicated concentrations. After 24 h, each bacterial culture was spot inoculated (10 μl/spot) on an agar plate and further grown overnight.

After construction of sequencing libraries, paired-end sequencing on the Illumina Hi-Seq X platform was performed then raw sequencing reads with high quality were used to assemble genomes. We constructed the genomes of E. hormaechei KHUD 001 and KHUD 002 using SPAdes (version 3.15.3) with default parameters, and created 25 and 43 scaffolds, respectively. The NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016) was used for gene annotation of the genomes. E. hormaechei KHUD 001 genome is 4,836,070 bp with G + C content of 55.36% (Table 1). A total of 4,476 protein-coding genes, 6 rRNAs, and 74 tRNAs were identified. E. hormaechei KHUD 002 also consists of a similar-sized chromosome (4,848,366 bp with G + C content of 55.33%). We annotated 6 rRNAs, 72 tRNAs and 4,503 functional proteincoding genes from this strain. Both genomes were identified to carry several genes associated with multidrug efflux pump systems, such as the major facilitator superfamily (MFS) and resistance-nodulation-cell division (RND) (Table 1). The strains have been deposited in the Korean Collection for Type Cultures (KCTC 92564, KCTC 92565) for future reference and research.

Table 1. Genome features of E. hormaechei KHUD_001 and KHUD_002

Attribute	KHUD_001	KHUD_002
Genome size (bp)	4,836,070	4,848,366
GC content (%)	55.36	55.33
No. of scaffolds	25	43
Total genes	4,624	4,649
Protein-coding genes	4,476	4,503
tRNAs	74	72
Complete rRNAs (5S)	1	1
Partial rRNAs (16S, 23S)	3, 2	3, 2
ncRNAs	5	5
Pseudogenes	63	63
Locus tags related to MFS	MTX57_01660, MTX57_05430, MTX57_07095, MTX57_08280, MTX57_08335, MTX57_11805, MTX57_13825, MTX57_13830, MTX57_17865	MY532_01665, MY532_05440 MY532_07105, MY532_08290, MY532_08345, MY532_10340, MY532_13835, MY532_13840, MY532_17870
Locus tags related to RND	MTX57_00590, MTX57_00595, MTX57_00775, MTX57_02335, MTX57_02340, MTX57_02345, MTX57_02690, MTX57_02695, MTX57_09485, MTX57_10380, MTX57_10385, MTX57_10825, MTX57_10830, MTX57_11275, MTX57_11280, MTX57_14165, MTX57_14170, MTX57_18000, MTX57_18005, MTX57_19390, MTX57_19400, MTX57_19405	MY532_00595, MY532_00600, MY532_00780, MY532_02340, MY532_02345, MY532_02350, MY532_02695, MY532_02700, MY532_09495, MY532_10865, MY532_10870, MY532_11315, MY532_11320, MY532_11760, MY532_11765, MY532_15915, MY532_15920, MY532_18005, MY532_18010, MY532_20200, MY532_20205, MY532_20215

Nucleotide sequence accession number

This Whole Genome Shotgun sequencing project for *Enterobacter hormaechei* KHUD_001 and KHUD_002 is available at NCBI GenBank under BioProject ID PRJNA821275 (BioSample SAMN27065044; KHUD_001 and SAMN27625051; KHUD_002).

적 요

본 연구는 플루오로퀴놀론에 내성을 보이는 두 가지 Enterobacter hormaechei 균주를 한국인 치주염환자의 치면세 균막에서 분리하여 유전체 서열을 분석하였다. 균주KHUD_001 및 KHUD_002의 유전체는 각각 4,836,070 bp (G+C 함량: 55.36%) 및 4,848,366 bp (G+C 함량: 55.33%)이었다. 두 유전체 모두 항미생물제제에 대한 저항성과 연관된 MFS와 RND와 같은 다중약물 유출 펌프를 암호화하는 유전자들을 포함하고 있다.

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Conflict of Interest

The authors have no conflict of interest to report.

Ethical Statement

This study was approved by the Ethics Committee of Kyung Hee University Dental Hospital (Approval number : KHD IRB 1605-5).

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