

Complete genome sequence of multidrug-resistant *Moraxella osloensis* NP7 with multiple plasmids isolated from human skin

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사람의 피부에서 분리한 다약제 내성이며 다수의 플라스미드를 갖는 *Moraxella osloensis* NP7 균주의 유전체 서열 분석

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Moraxella osloensis NP7 was isolated from human skin of a collage male and showed resistance to β -lactam and aminoglycoside antibiotics. Herein, we report the complete whole-genome sequence and gene annotations of *M. osloensis* NP7. It possesses single circular chromosome and seven plasmids. Chromosome is 2,389,582 bp in length with the G + C content of 43.9% and encodes 2,065 protein-coding genes. The combined seven plasmids are 654,202 bp in size with the average G + C content of 40.5% and code for a total of 667 protein-coding genes. The chromosome of NP7 strain contains four ribosomal RNA operon copies, one transfer-messenger RNA gene, forty-seven tRNA genes, three riboswitch genes and three CRISPR arrays. Additional CRISPR array is found in the plasmid pNP7-1. The genes conferring resistance to β -lactam and aminoglycoside antibiotics were predicted to reside in the plasmid pNP7-1.

Keywords: *Moraxella osloensis*, multidrug resistance, multiple plasmids, skin

Moraxella osloensis, a Gram-negative bacterium, is identified in diverse environments including human skin, soil, wet household surfaces and nematode (Kubota *et al.*, 2012; Cosseau *et al.*,

2016; Cardinale *et al.*, 2017). From human skin this species was isolated for their capability to catabolize alkylphenol polyethoxylates, a potential source of endocrine disrupting chemicals (Lim *et al.*, 2018). Even this species is frequently involved in human infectious diseases such as bacteremia; the treatment of the bacterial infection was not difficult due to their antibiotic susceptibility (Han and Tarrand, 2004; Dien Bard *et al.*, 2011). Here we firstly isolated a multidrug-resistant *Moraxella osloensis* NP7 from human skin. The genomic information of this organism will help us understand the genetic background for the antibiotic resistance when compared to the complete genome sequences of antibiotic-susceptible *M. osloensis* strains isolated from human skin.

M. osloensis strains NP7 (KCTC 52864) was isolated from the nose skin of a male college student. The swab sampling was directly streaked on minimal salts basal (MSB) medium containing 0.5% sodium pyruvate (Stanier *et al.*, 1966). Of ten *M. osloensis* strains isolated from different human skins, only NP7 strain showed growth on the medium containing 20 μ g/ml of each of ampicillin, streptomycin, kanamycin, or gentamicin. Ethical approval for subject sampling was granted by the Changwon National University ethics committee.

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Table 1. General genomic features of the *Moraxella osloensis* NP7

Feature	Chromosome	pNP7-1	pNP7-2	pNP7-3	pNP7-4	pNP7-5	pNP7-6	pNP7-7
GenBank accession	CP024443.2	CP024444	CP024445	CP024446	CP024447	CP024448	CP024449	CP024450
Number of contigs	1	1	1	1	1	1	1	1
Genome size (bp)	2,389,582	271,709	134,961	65,541	61,809	59,633	46,732	13,817
G + C content (%)	43.9	40.7	38.8	40.5	41.8	41.9	41.5	37.0
Number of protein-coding genes (CDS)	2,065	269	141	68	62	65	46	16
Number of t-RNA genes	47	0	0	0	0	0	0	0
Number of rRNA (5S, 16S, 23S) genes	12	0	0	0	0	0	0	0
Number of CRISPR arrays	3	1	0	0	0	0	0	0
Number of transfer - messenger RNA genes	1	0	0	0	0	0	0	0
Number of riboswitch genes	3	0	0	0	0	0	0	0

Total DNA of the cultured cells was purified using the phenol extraction method (Ausubel *et al.*, 1990). The genome of *M. osloensis* NP7 was sequenced based on the RS II platform (Pacific Biosciences) using 20-kb SMRTbell template libraries (National Instrumentation Center for Environmental Management [NICEM], Seoul National University). The obtained reads, with a 270-fold genome coverage, were assembled *de novo* using Hierarchical Genome Assembly Process (HGAP) 3.0. Complete genome sequence was further amended by bioinformatics analyses, as previously described (Lim *et al.*, 2014). Gene predictions and annotations were carried out by the NCBI using the Prokaryotic Genome Annotation Pipeline (Besemer *et al.*, 2001).

The complete genome of *M. osloensis* NP7 contains one circular chromosome and seven plasmids, named pNP7-1~pNP7-7. The genome statistics show in Table 1. In comparison with chromosome, the nucleotide sequences of the plasmids were least homologous to those of the plasmids identified from *M. osloensis* strains TT16, KSH, and YHS, which are also skin origins (Lim *et al.*, 2018). Previously, we have shown that TT16 and KSH strains have four plasmids and YHS strain has three plasmids. These results indicated that the flexibility of extra-chromosomal genetic elements is one of key factors responsible for *M. osloensis* survival in diverse environments (Kubota *et al.*, 2012; Lim *et al.*, 2018). These could be further proved that the plasmid pNP7-1 possesses genetic loci that encode the BRO family class A β -lactamase (NP7_09525) and the aminoglycoside *O*-phosphotransferases (NP7_09795, NP7_09960, and NP7_09965). *M. osloensis* strains TT16, KSH and YHS are susceptible to the antibiotics. The genomic information

of NP7 strain can be very useful to understand the *Moraxella osloensis* adaptations to diverse environments including human skin and acquisition of antibiotic resistance.

Nucleotide sequence accession number

The complete genome sequence of the *M. osloensis* NP7 strain was deposited in GenBank under the accession numbers CP024443.2 for chromosome and CP024444 to CP024450 for plasmids.

적 요

남자 대학생의 피부에서 분리한 *Moraxella osloensis* NP7는 베타-락탐과 아미노글리코사이드 항생제에 대해 내성을 보였다. 본 연구에서는 NP7 균주 유전체의 완전한 염기서열과 유전자 주석을 보고하고자 한다. NP7 균주는 원형 염색체와 7개의 플라스미드를 갖고 있다. 염색체는 43.9%의 G+C 함량을 갖는 2,389,582개의 염기쌍을 갖고 있으며, 단백질을 암호화하는 2,065개의 유전자를 보유하고 있다. 전체 플라스미드는 평균적으로 40.5%의 G+C 함량을 갖는 654,202개의 염기쌍을 갖고 있으며, 단백질을 암호화하는 667개의 유전자를 보유하고 있다. 염색체는 4개의 리보솜 RNA 오페론, 1개의 transfer-messenger RNA 유전자, 47개의 tRNA 유전자, 3개의 핵산스위치 유전자 그리고 3개의 CRISPR array를 포함하고 있으며, 1개의 CRISPR은 pNP7-1 플라스미드에 존재한다. 베타-락탐과 아미노글리코사이드 항생제에 내성을 부여하는 유전자는 pNP7-1 플라스미드에 존재하고 있다.

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