

Complete genome sequence of *Edwardsiella anguillarum* strain C-5-1 isolated from diseased Giant mottled eel (*Anguilla marmorata*) cultured in Korea[§]

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국내 양식 무태장어(*Anguilla marmorata*)에서 분리한 *Edwardsiella anguillarum* C-5-1의 전장 유전체 분석[§]

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Edwardsiella spp. is an important pathogen causing edwardsiellosis in aquaculture globally. *Edwardsiella anguillarum* has been recently described and is the more relevant pathogen in the eel farming industry. Here, we report the complete genome of the *E. anguillarum* strain C-5-1, isolated from a diseased giant mottled eel (*Anguilla marmorata*) cultured in Korea. The genome consists of a circular chromosome and one plasmid with 3,771 protein-coding genes, 25 rRNAs, 99 tRNAs, 6 ncRNAs, and 109 pseudogenes. Moreover, several genes for virulence and antimicrobial resistance, prophage regions, and CRISPR spacers were detected in the C-5-1 genome. Interestingly, *tet(D)* in this

plasmid is almost identical (> 99%) to other closely related bacteria found in Korea and Japan, suggesting that it could be associated with increasing antimicrobial resistance of *Edwardsiella* spp.. This genomic information will provide important insights for strategies against prevalent pathogens in the Korean aquaculture industry.

Keywords: edwardsiellosis, antimicrobial resistance, eel, prophage, *tet(D)*

The genus *Edwardsiella* belongs to the Hafniaceae family in Enterobacterales and comprises five valid species: *E. anguillarum*, *E. ictaluri*, *E. piscicida*, *E. hoshinae*, and *E. tarda* (Buján *et al.*, 2018). These are major bacterial pathogens causing edwardsiellosis in aquatic animals, responsible for economic losses in aquaculture globally (Park *et al.*, 2012; Soto *et al.*, 2012). These bacteria

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may cause zoonotic infections in warm-blooded animals and humans (Adepoju *et al.*, 2021; Pham, *et al.*, 2021). Among the five species, *E. tarda* has been well-studied as it has representative zoonotic pathogenicity (Michael and Abbott, 1993; Xu and Zhang, 2014): studies have shown that the species actually represents three genetically distinct taxa, and almost all the fish-pathogenic isolates were re-assigned as *E. anguillarum* and *E. piscicida* (Shao *et al.*, 2015; Buján *et al.*, 2018). *Edwardsiella anguillarum* is now considered a newly emerging aquatic pathogen, in aquaculture (especially in eel culture) and wild aquatic animals (Oh *et al.*, 2020). Extensive antibiotic usage in global aquaculture has resulted in antimicrobial resistance (AMR) to various agents, as observed in *Edwardsiella* isolates (Wimalasena *et al.*, 2019; Algammal *et al.*, 2022). Therefore, the identification of potential virulence factors and AMR-associated genes in *E. anguillarum* is urgently needed, as the mechanisms underlying them remain unclear. To date, only seven genome sequences for *E. anguillarum* (one complete and six drafts) are available in the GenBank database (accessed in December 2022), mostly isolated from diseased Japanese eels (*Anguilla japonica*). Here, we present the first complete genome sequence of *E. anguillarum* isolated from a diseased giant mottled eel (*Anguilla marmorata*) cultured in Korea.

The moribund juvenile giant mottled eels with petechial hemorrhage on the skin were collected from a private eel culture farm (Gyeonggi-do, Republic of Korea), and the representative C-5-1 strain was isolated from a liver abscess in the fish by culturing overnight on tryptic soy agar (Difco) at 25°C. Based on the *gyrB* sequence analysis (Pascual *et al.*, 2010), the isolated bacteria showed > 99% nucleotide identity with the *E. anguillarum* strains available in the GenBank database, including ET080813^T (NZ_CP006664.1), and was finally classified as *E. anguillarum*. For whole-genome sequencing, genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) and sequenced via a hybrid approach using the PacBio Sequel System (Pacific Biosciences), by constructing a 20-kb SMRTbellTM template library, and the HiSeq X-10 platform (Illumina), by preparing a DNA library using the TruSeq Nano DNA library prep kit (Illumina). The PacBio reads (599,706,396 bp; 69,247 reads) were assembled with the Microbial Assembly Application in the SMRT Link v8.0 (Ardui *et al.*, 2018). The Illumina paired-end reads (905,820,202 bp; 6,006,018 reads) were used for

accurate genome sequencing using Pilon v1.21 (Walker *et al.*, 2014). Genome annotation was performed using the NCBI prokaryotic genome annotation pipeline (<http://www.ncbi.nlm.nih.gov/books/NBK174280/>).

The C-5-1 genome comprised 4,293,127 bp (58.7% G + C content, 126× coverage), consisting of one chromosome (4,140,843 bp, 58.9% G + C content, 125× coverage) and one plasmid named p1_C-5-1 (152,284 bp, 53.3% G + C content, 156× coverage), encoding 3,771 protein-coding genes, 25 rRNAs, 99 tRNAs, 6 noncoding RNAs, and 109 pseudogenes (Table 1, Supplementary data Fig. S1). The genomic similarity between C-5-1 and other available type strains of *Edwardsiella* spp. was assessed using the Orthologous ANI tool software (Lee *et al.*, 2016) and the genome was most similar to *E. anguillarum* ET080813^T (99.96%) than to other *Edwardsiella* strains (Supplementary data Fig. S2). This was also validated by genome-based phylogeny using the Type (Strain) Genome Server (TYGS) (Meier-Kolthoff *et al.*, 2022) (Supplementary data Fig. S3).

Potential virulence-associated genes in the C-5-1 strain were compared with those of *Edwardsiella* spp. strains available in the GenBank database while additional virulence and AMR genes were screened as previously described (Lee *et al.*, 2018). Among those reported in *E. anguillarum* (Zhai *et al.*, 2021), genes encoding for the ABC transporter and flagellar assembly were detected in the C-5-1 chromosome. Several quorum sensing (QS)-related genes (*qseB*, *qseC*, *qseD*, and *LuxS/AI-2*), previously reported in *E. tarda*, have also been detected (Wang *et al.*, 2011; Park *et al.*, 2012). This suggests that C-5-1 has the

Table 1. The general features of *Edwardsiella anguillarum* strain C-5-1 genome

Features	Value
Genome size (bp)	4,293,127
Chromosome	4,140,843
Plasmid (p1_C-5-1)	152,284
G + C content (%)	58.7
No. of contigs	2
Plasmid	1
Total genes	4,010
Protein-coding genes	3,771
rRNAs	25
tRNAs	99
ncRNAs	6
Pseudogenes	109

potential ability to use QS to modify bacterial surface structures including flagella and fimbriae, increasing its survival rate in particular environmental niches.

Several AMR-related genes were detected in the C-5-1 chromosome and plasmid (Supplementary data Table S1). Interestingly, *tet(D)* (tetracycline efflux MFS transporter D) (locus_tag: MUN71_RS19745) was detected in the C-5-1 plasmid with a deduced amino acid sequence > 99% identical to those previously reported for *E. piscicida* isolated in Korea (Kim *et al.*, 2018), and to some strains of *Citrobacter* spp. and *Alteromonas* spp. isolated in Japan (Furushita *et al.*, 2003). This suggests that the gene can be transferred via plasmid between genera, as well as in *Edwardsiella* spp., and could be associated with Tet(D)-related antimicrobial resistance in the aquaculture industry.

Eight prophage regions were identified in the C-5-1 chromosome using the PHASTER (Phage Search Tool Enhanced Release) server (Arndt *et al.*, 2016), three of which were intact (score > 90) and five incompletes (score < 70) (Supplementary data Table S2). The length of intact prophage regions consisted of 17.6 kb (Region 2), 44.1 kb (Region 6), and 44.5 kb (Region 7). Prophages are known to be involved in evolving the pathogenicity of host bacteria including antibiotic resistance and adaptation to new ecological niches (Fortier and Sekulovic, 2013). In addition, several spacers derived from viral sequences were detected in their genome by CRISPRCasFinder (Grissa *et al.*, 2007). The analysis indicated that a total of 32 spacers, which were closely related to those from other *Edwardsiella* spp., *Citrobacter* spp., and *Salmonella* spp., were found in the C-5-1 chromosome. Based on these results, the strain C-5-1 could survive against various bacteriophage infections and might evolve into a more uncontrollable pathogen due to gene transfer carried out by prophages.

This is the first report on the genome of *E. anguillarum* in Korea. The results provide important insights into its genomic characteristics, which might lead to the development of strategies to control *E. anguillarum* prevalence in the Korean aquaculture industry.

Nucleotide sequence accession numbers

Edwardsiella anguillarum C-5-1 was deposited in the Korean Collection for Type Cultures (KCTC) under the accession

number KCTC 92163. The C-5-1 chromosome and plasmid were deposited in the DDBJ/END/GenBank under the accession numbers CP095163 and CP095164, respectively.

적 요

Edwardsiellosis는 수생 동물, 특히 양식 어류에 집단 폐사를 일으킬 수 있는 질병으로, *Edwardsiella* 속 세균 종들이 원인으로 알려져 있다. 현존하는 5종의 *Edwardsiella* spp. 중 *E. anguillarum*은 가장 최근에 분류된 종으로 국내·외 뱀장어 양식 산업에서 심각한 피해를 끼치고 있는 것으로 알려져 있다. 본 연구에서 우리는 국내에서 양식되는 무태장어(*Anguilla marmorata*) 중 병어로부터 *E. anguillarum* C-5-1을 분리하였으며, 해당 균주의 전장 유전체를 분석하였다. 분석 결과, *E. anguillarum* C-5-1 균주는 원형의 유전체(4,140,843 bp)와 하나의 플라스미드(p1_C-5-1; 152,284 bp)를 지니며, 3771개의 단백질 코딩 유전자로 구성됨을 확인하였다. 더불어 C-5-1 균주에서 다양한 병원성, 항생제 내성, prophage 영역 및 CRISPR spacer 관련 유전자들의 존재가 확인되었다. 흥미롭게도, p1_C-5-1 플라스미드에서 한국 및 일본 등에서 보고된 서열과 매우 상동성이 높은 *tet(D)* 유전자가 확인되었으며, 이는 국내 양식 분야에서 *Edwardsiella* spp.의 항생제 내성 전파와 밀접한 관련성이 있을 것으로 추정된다. 본 연구 결과는 국내 양식 어류에서 출현하는 잠재적 인수공통 감염을 지닌 *E. anguillarum*의 특성과 더불어 향후 해당 세균에 의한 감염증 제어 기법 발굴을 위한 기반 자료로 활용될 것이다.

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Conflict of Interest

The authors have no conflict of interest to report.

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