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Identification and characterization of an intervening sequence within the 23S ribosomal RNA genes of *Edwardsiella ictaluri*☆

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Abstract

Comparison of the 23S rRNA gene sequences of *Edwardsiella tarda* and *Edw. ictaluri* confirmed a close phylogenetic relationship between these two fish pathogen species and a distant relation with the 'core' members of the *Enterobacteriaceae* family. Analysis of the *rrl* gene for 23S rRNA in *Edw. ictaluri* revealed the presence of an intervening sequence (IVS) in helix-45. This new 98 bp IVS shared 97% nucleotide identity with *Salmonella typhimurium* helix-45 IVS. *Edw. ictaluri* helix-45 IVS was present in all *Edw. ictaluri* strains analyzed and in at least six *rrl* operons within each cell. Fragmentation of 23S rRNA due to IVS excision by RNase III was observed by methylene blue staining of ribosomal RNA extracted from *Edw. ictaluri* isolates. This is the first report of an IVS in the 23S rRNA gene of the genus *Edwardsiella.*

Keywords: *Edwardsiella*; Intervening sequences (IVS); *rrl* genes

Introduction

The genus *Edwardsiella* was first described in 1965 by Ewing et al. [11] to harbor a collection of 37 strains biochemically distinct from other taxa within the family *Enterobacteriaceae*. The proposed type species for the genus was *Edwardsiella tarda* and at that time comprised isolates of fecal origin from the USA. At about the same time, a Japanese group studying bacterial isolates from reptiles identified a distinct yet homogeneous group, referred to as the Asakusa group, within the *Enterobacteriaceae* family [28] which appeared to be very similar to the newly described *Edw. tarda*. It was not until 1980 that a second species, *Edw. hoshinae*, was added to the genus by Grimont et al. [13] who isolated it from reptiles and birds. Finally, Hawke et al. [14] added the last species to the genus, *Edw. ictaluri*, described from isolates recovered from diseased channel catfish (*Ictalurus punctatus* Rafinesque).

The phylogenetic position of the genus *Edwardsiella* within the family *Enterobacteriaceae* is somewhat unclear since few evolutionary studies including this genus have been attempted [1,2]. Nevertheless, DNA-DNA hybridization data pointed to *Serratia* as the closest genus to *Edwardsiella* at 20% similarity [17]. Other studies based on different signature sequences, such as $tRNA^{Leu}$ and 16S rRNA, confirmed a distant phylogenetic relationship between *Edwardsiella* and core members of the *Enterobacteriaceae* such as *Escherichia*

☆The GenBank accession numbers corresponding to partial sequences of the 23S rRNA genes of *Edwardsiella tarda* CECT 849, *Edw. ictaluri* CECT 885 and *Edw. ictaluri* EILO are DQ211093, DQ314205, and DQ211094, respectively.

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or *Salmonella* [12]. Phylogenetic comparisons between the three *Edwardsiella* species are also scarce. Panangala et al. [23] studied the interspecies and intraspecies differences between *Edw. tarda* and *Edw. ictaluri* based on the 16S-23S rRNA intergenic spacer region (ISR) and concluded both species were highly similar (>96% identity). To our knowledge, no formal ribosomal genes-based trees including *Edw. tarda* and *Edw. ictaluri* sequences have been published. At the intraspecific level, it is widely accepted that *Edw. tarda* presents a higher degree of intrinsic diversity than *Edw. ictaluri* [16,23]. Only by using the high resolution fingerprinting technique AFLP (Amplified Fragment Length Polymorphism) has it been possible to discriminate among *Edw. ictaluri* isolates [16].

Edw. ictaluri is the causal agent of enteric septicemia of catfish (ESC) and is responsible for great economic losses to the catfish industry across the world [25,31]. Only in the USA, where channel catfish production accounts for more than half of total aquaculture products, it is estimated that ESC costs the catfish industry $19 million yearly in direct fish losses [29,30]. Although a few non-ictalurid species (e.g. tilapia, Chinook salmon, and rainbow trout) are susceptible to experimental infection, *Edw. ictaluri* natural host range remains restricted to members of the *Ictaluridae* family, particularly channel catfish. On the contrary, *Edw. tarda* has shown a much broader host spectrum with the ability to infect fish from different families as well as reptiles and even humans, though is a minor source of disease in aquaculture. The pathogenic factors that made *Edw. ictaluri* a specialized catfish pathogen while *Edw. tarda* retained its broader spectrum are not known, nor are the phylogenetic relationships between these two pathogens.

The aim of this work was to expand our knowledge on the phylogenetic relationships between *Edw. tarda* and *Edw. ictaluri* by comparing their 23S rRNA gene sequence and to find new signature sequences for ESC detection. As a result of this study, a new intervening sequence (IVS) in the 23S rRNA of *Edw. ictaluri* was identified for the first time.

Material and methods

Bacterial strains and cultivation conditions

Twenty nine *Edw. ictaluri* strains, including the type strain CECT 885, along with five *Edw. tarda* strains and *Escherichia coli* K-12 strain were used in this study (Table 1). All strains were grown on brain heart infusion (BHI) (Becton, Dickinson and Company, Sparks, MD) broth or BHI agar (supplemented with 1.5% agar) at 28 °C, except for *E. coli* which was incubated at 37 °C. Stock cultures were maintained in 15% glycerol at −70 °C, and a single colony was isolated prior to use.

23S rDNA gene amplification, cloning and sequencing

Total bacterial DNA was extracted using the Qiagen DNeasy Tissue kit (Qiagen, Maryland, DE) following manufacturer's instructions. Two sets of universal primers 118 V 5′-CCGAATGGGGAAACCCA-3′ (positions 112–130 in *E. coli*), 1037R 5′-CGACAAGGAATTTCGCTAC-3′ (positions 1930–1948), 23_2F (5′-GGCGGCCGTAACTATAACG-3′) (positions 1901–1919), and 23_2R (5′-AGCCTCACGGTTCATTAGTACC-3′) (positions 2874–2895), complementary to highly conserved regions of eubacterial 23S rRNA genes were used to amplified the 23S rRNA gene from *Edw. ictaluri* CECT 885 and EILO and *Edw. tarda* CECT 849. PCR

Table 1. Strains used in the study

Species	Strain	Origin
Edw. ictaluri	CECT 885^T, 195, 196, 151, 218, 219, 1696, 1760, 1963, AL-93-75, AL-02-27, AL-93-58, ALG-03-278, ALG-03-190 ALG-03-161, ALG-03-278 ALG-03-275, ALG-03-277 ALG-03-192	Catfish, Alabama, USA
	S94-728, S94-1034, S94-715, S94-703, S94-827, S94-862, S94-872, S94-1051, S94-707	Catfish, Mississippi, USA
	EILO	Catfish, Thailand
Edw. tarda	CECT 849^T	Human feces
	1909, Flb, Flb2, 172,	Catfish, Alabama, USA
E. coli	K-12	Human feces

T, type strain.
CECT, Spanish Type Culture Collection.