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Short communication

Sequence variants of *Yersinia enterocolitica ystB* gene detected in wild animals in Poland

M. Pieczywek, A. Bancerz-Kisiel, A. Szczerba-Turek, W. Szweda

Department of Epizootiology, Faculty of Veterinary Medicine,
University of Warmia and Mazury, Oczapowskiego 13, 10-718 Olsztyn, Poland

Abstract

The purpose of the study was to analyze a part of the nucleotide sequences of *ystB* gene *Y. enterocolitica* strains isolated from wild animals. The material for the study consists of 30 *Y. enterocolitica* biotype 1A strains obtained from different wild animal species and belonging to different genotypes. Phylogenetic analysis of *ystB* nucleotide sequences belonging to four regular genotypes G1, G2, G3, G4 and to five groups of variations V1, V2, V3, V4, V5 revealed significant differences of *Y. enterocolitica* strains isolated from wild animals. The most phylogenetically distant were strains belonging to V5.

Key words: *Yersinia enterocolitica, ystB* gene, phylogenetic analysis

Introduction

Yersinia (Y.) enterocolitica is one of the most important foodborne pathogens, that can cause disease in humans and animals. Based on the specific biochemical features, *Y. enterocolitica* has been divided into six biotypes: 1A, 1B, 2, 3, 4, 5. Strains belonging to 1B and 2-5 are classically considered as pathogenic, while biotype 1A strains are commonly regarded as nonpathogenic (Bottone 2015). These strains do not carry the plasmid of *Yersinia* virulence (pYV) and most chromosomal virulence genes, such as *ail, ystA* and *myfA,* encoding respectively Ail (attachment-invasion locus), YstA (*Yersinia* stable toxin), and MyfA (mucoid *Yersinia* factor) (Stephan et al. 2013). Strains belonging to biotype 1A were repeatedly isolated from healthy patients, however clinical cases of yersiniosis caused by this biotype are also reported. Recent papers indicate even that the majority of *Y. enterocolitica* strains isolated from patients with diarrhea in Finland belongs to biotype 1A (Huovinen et al. 2010). Pathogenicity of *Y. enterocolitica* strains depends on plasmid and chromosomal virulence markers. One of the most genetically stable chromosomal virulence markers is *yst* gene, which encodes enterotoxins Yst, divided into YstI (A, B and C) and relatively recently discovered YstII. Classically, pathogenic *Y. enterocolitica* strains are able to produce enterotoxin YstA, while biotype 1A strains were reported to produce enterotoxin YstB, encoded by the *ystB* gene (Bancerz-Kisiel et al. 2017).

Correspondence to: A. Bancerz-Kisiel, e-mail a.bancerz-kisiel@uwm.edu.pl

Materials and Methods

The material for the study consisted of 30 *Y. enterocolitica* biotype 1A strains obtained from different wild animal species and belonging to different genotypes (Bancerz-Kisiel et al. 2017). Five strains from each genotype defined using HRM (High-resolution melting) method were randomly selected for the experiment and 10 variations were also examined. PCR analyses using new primer sequences *ystB*-1/*ystB*-2 (Platt-Samoraj et al. 2015) were performed to obtain *ystB* gene fragments with a length of 263 bp (base pair). The PCR conditions were: denaturation at 95°C for 5 min, then 40 cycles included 10 s denaturation at 95°C, annealing at 42°C for 30 s and elongation at 72°C for 10 s, for each cycle. The final elongation was performed at 72°C for 10 min. The amplicons were then purified using a Clean-up Purification Kit (A&A Biotechnology, Poland), and directly sequenced (Genomed S.A., Poland). Phylogenetic analysis was conducted using the freeware Computation Evolutionary Biology package MEGA version 5.2.1 (Tamura et al. 2011).

Results and Discussion

This is one of the first studies on the phylogenetic analysis based on nucleotide sequences of *ystB* genes *Y. enterocolitica* strains isolated from wild animals. Evolutionary relationships of 19 taxa of *ystB* gene are shown in Fig. 1. Phylogenetic analysis of randomly chosen nucleotide sequences belonging to four regular genotypes G1, G2, G3, G4 and to five groups of variations V1, V2, V3, V4, V5 revealed significant differences of *Y. enterocolitica* strains isolated from wild animals. The most phylogenetically distant were strains belonging to V5.

Nucleotide sequences of *ystB* genes assigned to G1 showed 100% identify with *Y. enterocolitica* DNA for *Yersinia* Heat-stable Enterotoxin Type B, complete cds (Acc. No. D88145), *Y. enterocolitica* (type O:5) YE53/03 complete genome (Acc. No. HF571988), and *Y. enterocolitica* strain isolated from beavers (Acc. No. KJ592623). Nucleotide sequences of *ystB* genes assigned to G3 showed 100% sequence identify with *Y. enterocolitica* strain isolated from beavers (Acc. No. KJ592626), as well as nucleotide sequences of *ystB* genes assigned to V2 (100% sequence identify with *Y. enterocolitica* strain Acc. No. KJ592627). The rest of *ystB* nucleotide sequences were defined for the first time, new sequence variants were published in National Center for Biotechnology Information (NCBI) (Acc. No. KM253270-KM253274, KM253276, KM253278--KM253280, KM253283-KM253285, KU198401--KU198402). No correlation was observed between the genotype and the species of animal from which the strain was isolated.

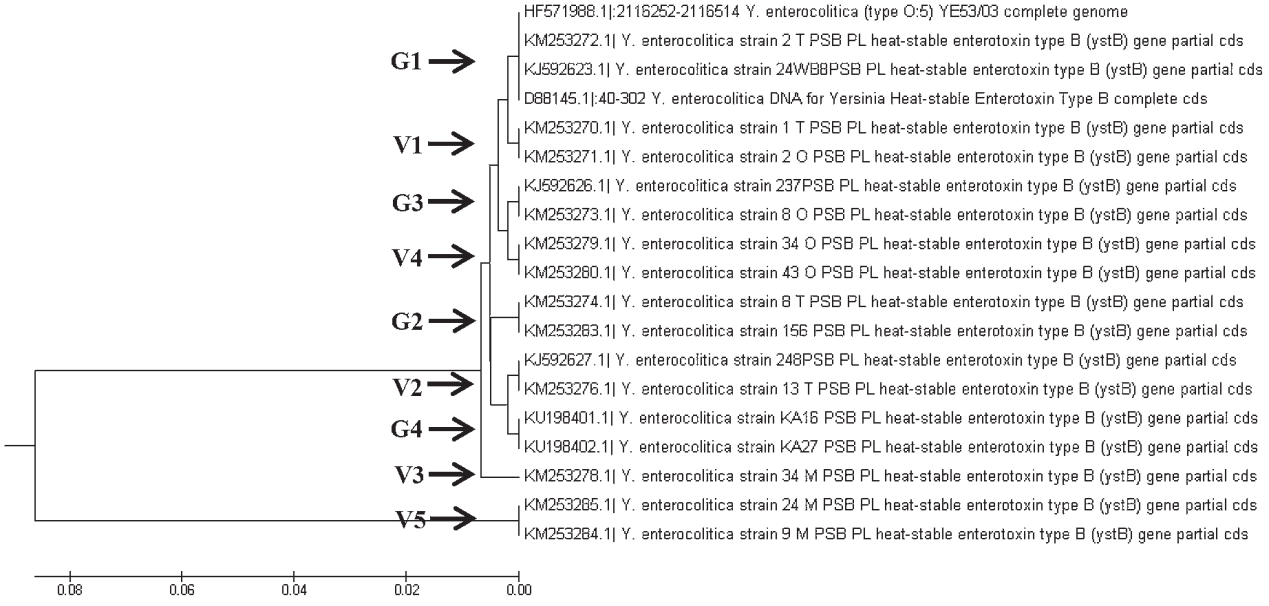

Fig. 1. Evolutionary relationships of taxa. The evolutionary history was inferred using the UPGMA method (Sneath and Sokal 1973). The optimal tree with the sum of branch length = 0.19884130 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The analysis involved 19 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 253 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

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