Food Control 96 (2019) 271–280

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

DNA sequences and predicted protein structures of *prot*6E and *sef*A genes for *Salmonella* ser. Enteritidis detection☆

Lijun Hu[a], Robert Stones[b], Eric W. Brown[a], Marc W. Allard[a], Li M. Ma[c], Guodong Zhang[a,*]

[a] Division of Microbiology, Office of Regulatory Science, Center for Food Safety and Nutrition, U.S. Food and Drug Administration, College Park, MD, USA
[b] Newcastle University, Newcastle upon Tyne, UK
[c] National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, USA

ARTICLE INFO

Keywords:
*prot*6E
*sef*A
Enteritidis
WGS
Protein structure

ABSTRACT

Genes *prot*6E and *sef*A are used as targets for detection of *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*Salmonella* ser. Enteritidis). We investigated variations in these genes across 64 different *Salmonella* ser. Enteritidis strains isolated from egg and chicken samples, then used Whole Genome Sequence (WGS) data to model the structures of their protein products. Isolates were sequenced using Illumina technologies. Based on the resulting phylogenetic tree, our isolates clustered in 2 distinct clades. All isolates carried *prot*6E and *sef*A. Comparative genomic analyses indicated two non-synonymous mutations (Glycine → Serine and Valine → Isoleucine) of *prot*6E in 11 isolates (9 egg samples, 2 chicken samples). However, SWISS-MODEL was unable to clearly model the protein structure of these two mutations. We identified one non-synonymous mutation (Valine → Glutamic Acid) in the *sef*A gene in 4 isolates from egg samples. The model for the protein structure of this mutant gene was clearly different from that of the other isolates studied herein. Circular maps of plasmid genomes from two PacBio platform-sequenced *Salmonella* ser. Enteritidis isolates revealed *prot*6E gene was located on the tail of the plasmid. Based on the biosynthesis of amino acids - Reference pathway in the KEGG pathway Database, the transition of amino acid from *sef*A Var. was a transversion from essential amino acid to non-essential amino acid, while that of *prot*6E Var.1 happened between the conditionally non-essential amino acid, and *prot*6E Var. 2 occurred between essential amino acids. Properties of these mutated amino acids, such as side-chain polarity or charge, may contribute to the occurrence and rate of mutations in *prot*6E and *sef*A. These insights can be used to improve detection methods for *Salmonella* ser. Enteritidis.

1. Introduction

As one of the most common serotypes of *Salmonella* outbreaks reported worldwide, *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*Salmonella* ser. Enteritidis) is an important zoonotic food-borne pathogen (Hendriksen et al., 2011). Outbreaks of *Salmonella* ser. Enteritidis have frequently been associated with eggs and poultry products (De Reu et al., 2006; Dewey-Mattia et al., 2017; Kimura et al., 2004; Wright, Richardson, Mahon, Rothenberg, & Cole, 2016). It is estimated that contaminated egg and poultry products contributed to approximately 64% and 18% of *Salmonella* ser. Enteritidis clinical cases, respectively (Gould et al., 2013; Painter et al., 2013). During 2006–2009, the percentage of persons infected with *Salmonella* ser. Enteritidis increased in the United States (Chai et al., 2012). A CDC report summarizing the laboratory-confirmed surveillance data on *Salmonella* infections in 2015 showed that 1388 *Salmonella* ser. Enteritidis infections have occurred among a total of 7719 *Salmonella* infections (CDC, 2017). In Canada, a threefold increase of the laboratory-confirmed *Salmonella* ser. Enteritidis infections has been reported from 2003 to 2009, the incidence rate (IR, per 100,000 persons) surging from 2.2 in 2003 to 6.2 in 2009 (Landry & Dutil, 2010; Nesbitt et al., 2012). In 2014, an outbreak of *Salmonella* ser. Enteritidis infections linked to egg consumptions caused over 350 illnesses in the United Kingdom, Germany, Austria, France and Luxembourg (Dallman et al., 2016). Obviously, *Salmonella* ser. Enteritidis has been a significant threat to public health around the world. To address this threat to public health, we need better tools for the rapid and accurate detection of this pathogen. Here we will investigate two potential molecular targets which

☆ † Mention of trade names or commercial products in the paper is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the U. S. Food and Drug Administration.

* Corresponding author. Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5001 Campus Dr., College Park, 20740, USA.
E-mail address: Guodong.Zhang@fda.hhs.gov (G. Zhang).

https://doi.org/10.1016/j.foodcont.2018.09.018
Received 19 July 2018; Received in revised form 14 September 2018; Accepted 16 September 2018
Available online 21 September 2018
0956-7135/ Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

Table 1
Salmonella ser. Enteritidis strains used in the study.

Isolates	Source	Location	Year
CFSAN057702	Eggs	Brazil	2000
CFSAN057880	Egg	Brazil	2016
CFSAN025700	Whole eggs	USA:IN	2012
CFSAN024840	Whole eggs	USA:MN	2012
CFSAN025717	Whole eggs	USA:AL	2012
CFSAN025708	Whole eggs	USA:NJ	2012
CFSAN024814	Whole eggs	USA:NH	2012
CFSAN024727	Poultry egg	Chile	2009
CFSAN024743	Poultry egg	Chile	2012
CFSAN017081	Frozen liquid egg	USA:GA	2011
CFSAN057651	Cooked quail eggs	Brazil	1995
CFSAN002042	Egg slurry	N/A	N/A
CFSAN057762	Pecked eggs	Brazil	2004
CFSAN057760	Pecked eggs	Brazil	2004
CFSAN057767	Pecked eggs	Brazil	2005
CFSAN057769	Pecked eggs	Brazil	2005
CFSAN057773	Pecked eggs	Brazil	2005
CFSAN057775	Pecked eggs	Brazil	2006
CFSAN057780	Pecked eggs	Brazil	2006
CFSAN057783	Pecked eggs	Brazil	2006
CFSAN057789	Pecked eggs	Brazil	2007
CFSAN057791	Pecked eggs	Brazil	2007
CFSAN057793	Pecked eggs	Brazil	2007
CFSAN057794	Pecked eggs	Brazil	2008
CFSAN057795	Pecked eggs	Brazil	2008
CFSAN057796	Pecked eggs	Brazil	2008
CFSAN057812	Pecked eggs	Brazil	2009
CFSAN032971	Raw egg whites	USA:IA	2012
CFSAN028530	Raw egg whites	USA:NY	2012
CFSAN030835	Raw egg whites	USA:CA	2012
CFSAN033541	Raw egg whites	USA:PA	2013
CFSAN030081	Raw whole eggs	USA:NJ	2012
CFSAN030086	Raw whole eggs	USA:IA	2012
CFSAN032962	Raw whole eggs	USA:IA	2012
CFSAN033543	Raw whole eggs	USA:OH	2012
CFSAN035276	Raw whole eggs	USA:NY	2012
CFSAN027378	Raw whole eggs	USA:UT	2012
CFSAN027394	Raw whole eggs	USA:NH	2012
CFSAN030823	Raw whole eggs	USA:OH	2012
CFSAN035291	Raw whole eggs	USA:NJ	2012
CFSAN034231	Raw whole eggs	USA:GA	2013
CFSAN034232	Raw whole eggs	USA:GA	2013
CFSAN035272	Raw whole eggs	USA:TX	2013
CFSAN027377	Raw egg yolks	USA:NJ	2012
CFSAN030066	Raw egg yolks	USA:NY	2012
CFSAN030067	Raw egg yolks	USA:IA	2012
CFSAN030097	Raw egg yolks	USA:IN	2012
CFSAN030496	Raw egg yolks	USA:GA	2012
CFSAN030816	Raw egg yolks	USA:NJ	2012
CFSAN030839	Raw egg yolks	USA:WA	2012
CFSAN030852	Raw egg yolks	USA:NY	2012
CFSAN032958	Raw egg yolks	USA:NJ	2012
CFSAN032959	Raw egg yolks	USA:NJ	2012
CFSAN032964	Raw egg yolks	USA:NY	2012
CFSAN032970	Raw egg yolks	USA:IA	2012
CFSAN035289	Raw egg yolks	USA:NJ	2012
CFSAN035308	Raw egg yolks	USA:AL	2012
CFSAN035309	Raw egg yolks	USA:IA	2012
CFSAN034151	Raw egg yolks	USA:GA	2013
CFSAN057837	Chicken	Brazil	1990
CFSAN057838	Chicken	Brazil	1990
CFSAN057841	Chicken	Brazil	1993
CFSAN057814	Chicken	Brazil	2010
CFSAN058030	Chicken	USA:NJ	2014

AL, Alabama; CA, California; GA, Georgia; IA, Iowa; IN, Indiana; MN, Minnesota; NH, New Hampshire; NJ, New Jersey; NY, New York; OH, Ohio; PA, Pennsylvania; TX, Texas; UT, Utah.

may be indicative for the presence of *Salmonella* ser. Enteritidis.

Specifically, the genes *prot*6E and *sef*A have been used as targets for molecular detection of *Salmonella* ser. Enteritidis (Malorny, Bunge, & Helmuth, 2007; Seo, Valentin-Bon, Brackett, & Holt, 2004). Gene *prot*6E is located on a 60 kb virulence plasmid, and may encode a unique surface fimbriae that is specific to *Salmonella* ser. Enteritidis (Clavijo, Loui, Andersen, Riley, & Lu, 2006). The *Sef*A gene was first described in *Salmonella* ser. Enteritidis strains, and later found to be unique to *Salmonella* serogroup D serovars, including Gallinarum, Pullorum, Dublin, Rostock, Seremban, Typhi, etc. (Malorny et al., 2007). The *sef*A gene is located on the chromosome, encoding for *sef*A, a fimbrin, which is the structural subunit of SEF14 fimbriae (Clouthier, Müller, Doran, Collinson, & W.W., 1993). In terms of exclusivity, using *prot*6E gene has an advantage over *sef*A, because *prot*6E is only found in *Salmonella* ser. Enteritidis. However, *prot*6E is located on a plasmid. There are rare cases in which that plasmid is missing from a given *Salmonella* ser. Enteritidis strain. Therefore, relying on *prot*6E would result in a false negative result ocassionally. (González-Escalona, Zhang, & Brown, 2012; Malorny et al., 2007). Only limited information is currently available about the precise mechanisms and functions of these two genes. And few reports have been published describing the extent of their genetic variation and the effects of those variations upon resulting protein structures (Agron et al., 2001; De Medici et al., 2003; Malorny et al., 2007; Ravan & Yazdanparast, 2012; Techathuvanan, Draughon, & D'SOUZA, 2010; Woodward & Kirwan, 1996; Yang et al., 2010).

Advances in Whole Genome Sequencing (WGS) for foodborne pathogens allows us to explore these genes and their products with much greater precision than previous methods. WGS data can be easily obtained to determine the pathogen relatedness (identifying, classifying, and comparing pathogens), describe the genetic evaluation and metabolic pathways, and identify antibiotic resistance markers. For these reasons, WGS is increasingly being used in foodborne pathogen research, in combination with new bioinformatics tools (Gilchrist, Turner, Riley, Petri Jr., & Hewlett, 2015; Köser CU et al., 2012). Among these tools are automated systems such as SWISS-MODEL, which can derive protein structures based on WGS data and homology modeling (Biasini et al., 2014). These predicted structures can help establish the likely function of a protein product, the mechanisms by which it may function, and consequences of mutations to that protein.

The present study was initiated to analyze the two genes (*prot*6E and *sef*A gene) frequently used for *Salmonella* ser. Enteritidis detection by WGS, and model their protein structures by SWISS-MODEL, which will be very beneficial to design new nucleic acid-based detection methods for *Salmonella* ser. Enteritidis, and to explain how it differs from other *Salmonella* serovars in food products.

2. Materials and methods

2.1. Salmonella ser. Enteritidis isolates, culture conditions, and nucleic acid extraction

We analyzed 64 *Salmonella* ser. Enteritidis isolates, collected from egg (n = 59) and chicken (n = 5) samples in the years 1995–2014, in this study (Table 1). All isolates were from the collection of Division of Microbiology (DM), Office of Regulatory Science (ORS), at the Center for Food Safety and Applied Nutrition (CFSAN), of the U.S. Food and Drug Administration (FDA). Two of these isolates had originally been obtained from Chile, 22 isolates had been obtained from Brazil, and 1 isolate was from an unknown location. The remaining 39 isolates were from United States. The information of all isolates can also be searched from the NCBI Pathogen Detection system (https://www.ncbi.nlm.nih.gov/pathogens/). All isolates were cultured overnight at 37 ± 2 °C in tryptic soy broth (Becton Dickinson, Franklin Lakes, NJ, USA), then genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA).

2.2. Genome sequencing and analysis

All isolates were sequenced on an Illumina MiSeq platform following the manufacturer's instruction. The readings were assembled *de novo* using CLC Genomics Workbench v9.5.3 (Qiagen Bioinformatics,

Download English Version:

https://daneshyari.com/en/article/11030347

Download Persian Version:

https://daneshyari.com/article/11030347

Daneshyari.com