



Short Communication

Comparative genomic analysis of *Salmonella enterica* subsp. *enterica* serovar Weltevreden foodborne strains with other serovarsK. Brankatschk^a, J. Blom^b, A. Goesmann^b, T.H.M. Smits^{a,*}, B. Duffy^a^a Agroscope Changins-Wädenswil ACW, Plant Protection Division, Wädenswil, Switzerland^b CeBiTec, University of Bielefeld, Bielefeld, Germany

ARTICLE INFO

Article history:

Received 18 August 2011

Received in revised form 14 December 2011

Accepted 29 January 2012

Available online 3 February 2012

Keywords:

Carbohydrate metabolism

CRISPR

Type VI secretion systems

Food safety

Salmonella Weltevreden

ABSTRACT

Salmonella enterica subsp. *enterica* serovar Weltevreden is a dominant serovar associated with foodborne gastroenteritis in South-East Asia and emerging in Europe associated with fresh vegetables. Here we compared the genome of strain 2007-60-3289-1 linked to an alfalfa sprout outbreak in Scandinavia with a *S. Weltevreden* strain isolated from scallops in the USA and with other *S. enterica* serovars. A unique plasmid pSW82 was identified for *S. Weltevreden* carrying a two-component type II non-ribosomal peptide synthase/polyketide synthase. Analysis of all available complete *S. enterica* genomes identified differences for presence of type VI secretion systems and carbohydrate metabolic pathways. Differential transcription thereof was observed when *S. Weltevreden* strains were grown *in vitro* or on sprouts.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Salmonella enterica is a major cause of human gastroenteritis globally, with many of the over 2,500 serovars recognized based on somatic (O) and flagellar (H) antigens having been linked to foodborne disease. *S. enterica* subsp. *enterica* serovars Typhimurium and Enteritidis are predominant serovars which have broad host ranges infecting and colonizing diverse animal hosts (Herikstad et al., 2002). While a few serovars have more restricted host-ranges, for example *S. gallinarum* infecting chicken (Shivaprasad, 2000), many serovars have preferred primary host reservoirs (e.g., *S. Choleraesuis* infecting swine (Chiu et al., 2005)) but are efficient opportunistic colonizers of a wide variety of habitats and opportunistically infect humans.

A majority of foodborne disease is traceable to consumption of contaminated animal products (i.e., meat, seafood, poultry), but the epidemiological importance of plant products contaminated pre- or post-harvest (e.g., exposure to manure, sewage effluent, inappropriate handling during processing) is an increasing concern (Crook et al., 2003; Himathongkham et al., 1999; Horby et al., 2003). *S. Weltevreden* has long been a major problem associated with meat products in South-East Asia (Bangtrakulnonth et al., 2004; Learn-Han et al., 2008; Sood and Basu, 1979), and is an emerging serovar associated with

meat and plant products in Western countries. *S. Weltevreden* was linked for the first time to plant products having been traced as the cause of a recent outbreak of gastroenteritis in Scandinavia (Norway, Denmark and Finland) resulting from consumption of contaminated alfalfa sprouts (Emberland et al., 2007).

Complete genomes of *Salmonella* serovars with different host ranges are available and some have been compared on the genome level (Chiu et al., 2005; Sabbagh et al., 2010; Thomson et al., 2008). Comparison of *Salmonella* serovar genomes resulted in high synteny especially within core regions, which include most of the *Salmonella* pathogenicity islands (SPI). Differences were mainly found for presence or absence of phages, serovar specific SPIs and recombination of pseudogenes potentially responsible for host adaptation (Holt et al., 2009). However, comparative genomics has been limited generally to pair-wise comparisons and evaluation of clinical origin *Salmonella* serovars. A larger grouping of *Salmonella* serovars has been compared at the single gene (cluster) level, principally examining virulence factors (Blondel et al., 2009; Kröger and Fuchs, 2009). Comparisons of food safety relevant *Salmonella* isolates from plant and animal products that may shed light on host-range and habitat determinants are lacking.

The objective of this study was to provide a detailed analysis of the genome of an alfalfa sprout *S. Weltevreden* strain 2007-60-3289-1 (Arthurson et al., 2010; Brankatschk et al., 2011), compare this with the draft genome of a scallop *S. Weltevreden* strain SL484 and further compare the vegetable strain genome with complete sequences of other available *S. enterica* subsp. *enterica* serovar genomes. Our aim was to identify genetic features that may be involved in differential host range and unique serovar-specific behavior including virulence.

* Corresponding author at: Research Station Agroscope Changins-Wädenswil ACW, Division of Plant Protection, Postfach, Schloss, CH-8820 Wädenswil, Switzerland. Tel.: +41 44 783 6189; fax: +41 44 783 6305.

E-mail address: theo.smits@acw.admin.ch (T.H.M. Smits).

2. Materials and methods

2.1. Strains, growth media and conditions

All *S. enterica* subsp. *enterica* strains were grown on Luria–Bertani medium (LB) for extraction of genomic and plasmid DNA. *S. enterica* subsp. *enterica* serovar Weltevreden 2007-60-3289-1 (Arthurson et al., 2010) and SL484 (obtained from the *Salmonella* Genetic Stock Centre (University of Calgary, Calgary, Canada)) were grown on LB and on M9 minimal media (Sambrook et al., 1989) with 10 mM glucose or mannitol as a sole source of carbon for mRNA extraction. *S. Dublin* 2229 and *S. Typhimurium* LT2 were grown on M9 minimal media with 10 mM mannitol as a sole source of carbon for total RNA extraction. Cells were harvested during exponential ($OD_{600} = 0.4$) or in the stationary phase (overnight culture) and diluted to OD_{600nm} of 0.1 (approximately 0.7×10^8 cfu/ml) for extraction. For sprout cultures, *S. Weltevreden* strains 2007-60-3289-1 or SL 484 were pregrown over night, centrifuged and diluted to 10^6 cfu/ml in sterile deionised water. Five days old alfalfa sprouts (1.5 g) were inoculated with 4 ml of this suspension containing either *S. Weltevreden* strain. After 4 and 24 h incubation at 21 °C, 1 ml of culture liquid was collected, centrifuged and the pellet was used for total RNA extractions. For comparative studies, *S. Weltevreden* 2007-60-3289-1 and SL484 were grown on BIOLOG MicroPlates (Biolog, Inc., Hayward, CA, USA) for identification of anaerobic (AN) and aerobic Gram-negative (GN2) bacteria. Carbon sources that were utilized differently from both strains were added at 10 mM to M9 minimal media, inoculated separately with both strains and grown in a total volume of 200 µl using a Bioscreen C (Oy Growth Curves Ab Ltd., Raisio, Finland) for 48 hours at 21 °C.

2.2. Susceptibility test

Susceptibility to microbial agents was performed as screening of breakpoint concentrations following CLSI (Clinical and Laboratory Standards Institute) guidelines with one modification, using liquid LB broth in place of Mueller Hinton agar. The strain was exposed to 2 different concentrations that show whether the strain is susceptible or resistant to an antibiotic (Table S1). In 20 ml broth the strain was shaken in an incubator over night at 37 °C. The following antibiotics were used for susceptibility test: kanamycin, vancomycin, streptomycin, tetracycline and polymyxin B.

2.3. Molecular methods

Genomic DNA for sequencing was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). For plasmid DNA isolation, the NucleoBond® PC100 Plasmid DNA Purification Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) was applied. Amplification of genes specific for plasmid pSW82 and selected features followed a standard PCR protocol. Each 20 µl PCR reaction contained 10 µl of AccuStart™ Taq DNA Polymerase mix (Quanta BioSciences™, Gaithersburg, MD, USA); 2 µl of each primer (20 µM), 1 µl of DNA template and 5 µl water. PCR was performed with following conditions: initial denaturation at 95 °C for 5 min followed by 35 cycles of 95 °C for 45 s, 45 s at the respective annealing temperature (Table 1), 72 °C for 45 s, and a final extension at 72 °C for 10 min. Primer sets for target genes are shown in Table 1.

Extraction of total RNA from pellet of cultures grown in liquid media was done using NucleoSpin® RNA II (Macherey-Nagel, Düren, Germany). Extraction of total RNA from sprout supernatant was done using innuPREP Plant RNA kit (Analytik Jena, Jena, Germany). After extraction, remaining DNA was removed using DNase I (Fermentas, Thermo Scientific, Waltham, MA, USA) following the manufactures instructions. Presence of residual DNA was assayed by PCR using

Table 1

Primers designed for detection of plasmid pSW82 and expression of specific features in genome of *S. Weltevreden* 2007-60-3289-1.

Primer	Feature ^a	Locus tag	Sequence (5' → 3')	Annealing temperature (°C)
Plasmid_F		SENTW_5581	GTGTGTGTGCTACTCAGG	60
Plasmid_R			GGCACTGTACGGAATAGG	
T6SS_1_F	1	SENTW_0234	ACCGAACAGATCAAACTG	60
T6SS_1_R			TTGCAGTACCGAGGGGG	
T6SS_2_F	2	SENTW_1071	ATCGACGCCGTATCAG	57
T6SS_2_R			TTTTGAACATATGCGC	
A_F	7	SENTW_0550	AATACCACGCCGATCTG	60
A_R			GCTCAGCGGGTGTAA	
B_F	8	SENTW_1582	CTGTCTCAAGCATCCG	60
B_R			ATCCATCACCGCTGTGG	
C_F	9	SENTW_3196	ATCCCCATGTTCTTGG	57
C_R			GACTTTCGGTTCCG	

^a Feature number refers to Fig. 2.

16S rRNA gene-specific primers 63 F and 1389R (Marchesi et al., 1998). For reverse transcriptase PCR (RT-PCR), the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas, Thermo Scientific, Waltham, MA, USA) and gene-specific reverse primers were used, followed by standard PCR amplification of the target genes. As a positive control, the housekeeping gene *invA* (Fukushima et al., 2003) was included in all RT-PCR experiments.

2.4. Genome analysis software

Routine sequence manipulations were completed using several subroutines of the LASERGENE package (DNASTAR, Madison, WI, USA). Comparative genome analysis with the genome sequences of 17 *S. enterica* subsp. *enterica* serovars (Table 2) was done using EDGAR (Blom et al., 2009) with the settings as described previously (Smits et al., 2010), with the exception that new features were added to support multiple replicons per organism. For visualization, comparative analysis of the genome sequences was performed using Mauve (Darling et al., 2004) in the progressive mode. Analysis of unknown non-ribosomal peptide synthase/polyketide synthase was done using the PKS_NRPS analysis website (<http://nrps.igs.umaryland.edu/nrps/>).

Table 2

Genome sequence statistics for genomes of *Salmonella enterica* serovars used for comparative genomics analysis.

Serovar	NCBI project	Genome size (bp)	CDS	G + C content	Plasmid(s)
Weltevreden	61197	4,926,150	4,858	52.1	pSW82
2007-60-3289-1					
Weltevreden SL484	20591	5,047,463	5,133	52.0	-
Dublin CT_02021853	19467	4,842,908	4,514	52.0	pCT02021853_74
Newport SL254	18747	4,827,641	4,911	52.0	pSL254_3, pSN254
Enteritidis P125109	30687	4,685,848	4,423	52.0	-
Heidelberg SL476	20045	4,888,768	4,976	52.0	pSL476_3; pSL476_91
Gallinarum 287/91	30689	4,658,697	4,274	52.0	-
Typhimurium LT2	241	4,857,432	4,620	52.0	pSLT
Agona SL483	20063	4,798,660	4,884	52.0	unnamed
Choleraesuis SC-B67	9618	4,755,700	4,667	52.0	pSCV50, pSC138
Paratyphi A AKU_12601	30943	4,581,797	4,411	52.0	-
Paratyphi A ATCC 9150	13086	4,585,229	4,402	52.0	-
Paratyphi B SPB7	27803	4,858,887	5,771	52.0	-
Paratyphi C RKS4594	20993	4,833,080	4,829	52.0	pSPCV
Schwarzengrund	19459	4,709,075	4,802	52.0	pCVM19633_4, pCVM19633_110
CVM19633					
Typhi CT18	236	4,809,037	4,707	52.0	pHCM1/2
Typhi TY2	371	4,791,961	4,641	52.0	-

Download English Version:

<https://daneshyari.com/en/article/4367562>

Download Persian Version:

<https://daneshyari.com/article/4367562>

[Daneshyari.com](https://daneshyari.com)