Identification and characterization of Yersinia enterocolitica *strains isolated from pig tonsils at slaughterhouse in Central Italy*

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Summary
Yersinia enterocolitica causes foodborne disease in humans and infections are usually acquired from contaminated raw or undercooked pork. Pigs are considered the primary reservoir of human pathogenic bio-serotypes. A total of 376 tonsil tissue samples collected after evisceration and cutting from pig carcasses were tested for *Yersinia enterocolitica*. Animals came from an abattoir located in the Abruzzo region, Italy. *Yersinia enterocolitica* was isolated from 35 out of 376 (9.31%) samples. A total of 47 strains were isolated, the prevalent bio-serotype was 4/O:3 (95.74%), followed by bio-serotype 4/O:9 (2.13%), and 3/O:9 (2.13%). When characterized by DNA microarray, all strains clustered into 2 main groups. The bigger group was characterised by the presence of plasmid genes of the secretion apparatus as well as by the genes for the flagellum transport machinery, while the smaller group was characterised only by genes for the flagellum transport machinery. The high frequency of the pathogenic biotype 4/O:3 able to infect humans and considered an emerging zoonotic pathogen confirms the role of pigs as natural reservoir. Since there is no official data on *Yersinia enterocolitica*, it is difficult to assess the implications of this food pathogen for public health. A monitoring program should be implemented for contamination in food in order to assess the risk for the consumer linked to raw or undercooked pork products.

Identificazione e caratterizzazione di ceppi di Yersinia enterocolitica *isolati da tonsille di maiale in un mattatoio del centro Italia*

Parole chiave
Bio-sierotipo,
Geni di virulenza,
Maiale,
Microarray,
Tonsille,
Yersinia enterocolitica.

Riassunto
Yersinia enterocolitica, responsabile di tossinfezioni alimentari negli esseri umani, viene trasmesso principalmente da carne di maiale cruda o poco cotta. I suini costituiscono il principale serbatoio di ceppi patogeni per gli esseri umani. In questo studio è stata indagata la presenza di *Yersinia enterocolitica* in 376 campioni di tessuto prelevati, dopo taglio ed eviscerazione, dalle tonsille di altrettante carcasse di suino provenienti da un unico mattatoio in Abruzzo, Italia. Trentacinque su 376 campioni (9,31%) sono risultati positivi a *Yersinia enterocolitica* per un totale di 47 ceppi isolati. Il bio-sierotipo principale è risultato 4/O:3 (95,74%) seguito dal bio-sierotipo 4/O:9 (2,13%) e dal 3/O:9 (2,13%). Tutti i ceppi sono stati caratterizzati mediante DNA microarray e suddivisi in 2 gruppi: al gruppo più grande appartenevano i ceppi caratterizzati dalla presenza dei geni plasmidici dell'apparato di secrezione e dei geni del meccanismo di trasporto flagellare, mentre al gruppo più piccolo appartenevano i ceppi caratterizzati solo dalla presenza dei geni del meccanismo di trasporto flagellare. L'alta frequenza riscontrata del biotipo patogeno 4/O:3, capace di infettare gli esseri umani e considerato un agente zoonotico emergente, conferma il ruolo dei suini come serbatoio naturale. Per valutare il rischio del consumatore in seguito all'assunzione di prodotti a base di carne di suino cruda o poco cotta è necessario implementare un programma di monitoraggio sulla contaminazione degli alimenti da *Yersinia enterocolitica*, poiché attualmente non esistono dati ufficiali.

Introduction

Yersiniosis represents the third most frequent zoonosis in the European Union. Human infection is usually acquired from contaminated food, particularly raw or undercooked pork. Pigs are considered the primary reservoir of human pathogenic types (Fredriksson-Ahomaa *et al.* 2009). *Yersinia enterocolitica* is a gastrointestinal pathogen that usually occurs in young individuals. Frequent symptoms are diarrhoea, terminal ileitis, mesenteric lymphadenitis, and pseudo-appendicitis (Bottone 1997, Bottone 1999). *Yersinia enterocolitica* strains are classified into 6 biotypes (1A, 1B, 2, 3, 4, and 5) and more than 70 serotypes. Bio-serotypes associated with human yersiniosis usually belong to 1B/O:8, 2/O:9, 3/O:3, 4/0:3, and 2/O:5, 27 (Fredriksson-Ahoma and Korkeala 2003, Prentice *et al.* 1991). Pathogenic strains carry the pYV virulence plasmid that encodes a sophisticated secretion systems T3SS, named Ysc (Von Tils *et al.* 2012, Cornelis, 2002) and a variety of type III secreted effector proteins called Yops (*Yersinia* outer proteins). The Yops and the T3SS have been intensively investigated and their manipulating function on the host immune system is well-characterised (Cornelis and Wolf-Watz 1997, Trosky *et al.* 2008). Highly pathogenic strains which carry the pYV virulence plasmid and the high pathogenicity island (HPI) belong to biotype 1B, which is mainly isolated in North America and in Poland (Bottone 1999, Gierczyński *et al.* 2009). In Europe, Canada, Japan, and USA bioserotype 4/O:3 is the most common (Fredriksson-Ahomaa *et al.* 2011, Gierczyński *et al.* 2009, Poljak *et al.* 2010, Paixão *et al.* 2013). Besides, biotype 2-5, weakly pathogenic strains were also found in Europe and Japan (Fuchs *et al.* 2011). Biotype 1A strains are usually considered not virulent because of the absence of the pYV plasmid and chromosomal virulence genes, such as *ail, myfA, ystA*, and the *ysa* locus (Tennant *et al.* 2003, Robins-Browne *et al.* 1989, Foultier *et al.* 2002).

Regarding the consumption of meat, pork is the most eaten meat in Europe (Devine 2003) and the European pig farms are the largest in the world, after the Chinese (Institute Technique du Porc 2003). Control risk of zoonotic disease associated with the consumption of pork is therefore of major importance. Slaughtering procedures may offer many opportunities for the contamination of pork products. In this animal, the infection originates from contamination of local farming, in which bacteria can survive for a long time, and where there is probably faecal, water, and feed contamination. Pigs are particularly vulnerable in the hours immediately prior to slaughtering, as lairage stress, overcrowding, hunger, and subsequent coprophagia may facilitate the onset of infections. Zoonotic bacteria, such as *Yersinia enterocolitica*, are spread to the carcass surface mainly from carrier animals during evisceration (Laukkanen *et al.* 2008, Nesbakken *et al.* 2003, Van Damme *et al.* 2013). Strains of *Yersinia enterocolitica* capable of infecting humans are primarily isolated at tonsil level, mostly from the palatine tonsils, given that this pathogen has a strong tropism for the lymphoid tissue (Balada-Llasat and Mecsas 2006, Kapperud 1991). The contamination of pluck sets with tonsil may occur during slaughtering process (Fredrikson-Ahomaa *et al.* 2001a, Fredrikson-Ahomaa *et al.* 2001b).

Currently, in Europe no surveillance plan or microbiological criteria are applied for *Y. enterocolitica*. Moreover, little information is available regarding the distribution and the transmission of *Y. enterocolitica* among the slaughtered pigs in Italy, if we except data reported by Martinez and colleagues (Martinez *et al.* 2011) and Bonardi and colleagues (Bonardi *et al.* 2013). The present study was performed on samples collected from pigs slaughtered for meat consumption in an abattoir located in the Abruzzo region (Italy), with the aim to detect the frequency and bio-serotype distribution of *Y. enterocolitica* found in carcasses released for consumption in this geographic area at the time of the investigation. Two isolation techniques were applied: selective enrichment according to the International Standard Organization (ISO 10273:2003) and direct plating method as reported in surveillance monitoring program (EFSA Journal 2009). Furthermore, the isolates were characterised by an oligonucleotide microarray to detect the presence of virulence genes.

Materials and methods

Sample collection

From February to April 2011, a total of 376 pig carcasses were sampled for the presence of *Y. enterocolitica* in tonsil tissues in a slaughterhouse located in Abruzzo Region, Central Italy, details on the number of farms and pigs tested for each sampling day are reported in Table I. The collection of samples was performed after evisceration directly from the plucks, according to technical specifications proposed for the harmonised monitoring and reporting of *Y. enterocolitica* in slaughtered pigs in the Directive 2003/99/EC (EFSA Journal 2009). Samples were collected in sterile containers and then transported to the laboratory, stored at 4 ± 2 °C and analysed within 24 hours.

Statistical analysis

The 95% confidence intervals (CI) of the distribution

frequency of positive results were calculated using a Bayesian approach (Sivia 1996) with Beta distribution β(n+1;n-s+1), where n is the total number of tested samples and s are the tested positive samples.

Isolation of *Yersinia enterocolitica*

All strains were isolated using selective enrichment according to the International Standard Organization (ISO 10273:2003). For direct plating 1 ml of the tonsil homogenate was spread on 3 plates of cefsulodin-irgasan-novobiocin (CIN agar, Biolife, Milan, Italy), the plates were then incubated at 30°C for 24 hours to 48 hours and then checked for typical colonies (EFSA Journal 2009). Typical colonies were streaked on blood agar and after incubation at 30°C for 24 hours, they were tested for oxidase activity with a commercial kit (oxidase test DrySlide, BD BBI, Sparks, MD, USA). The same colonies were subcultured on Kligler agar after incubation at 30°C for 24 hours to 48 hours and analysed for the fermentation of glucose, lactose, and production of hydrogen sulphide. In addition, species identification was performed using API 20E (BioMérieux, Merçy L'Etoile, France) according to manufacturer's protocol.

Biotyping and serotyping

Yersinia enterocolitica was biotyped for its ability to ferment xylose and trehalose. These reactions were detected using Sugars Fermentation Tests (Liofilkem, Roseto degli Abruzzi, Italy). Indole production was tested by Indolo Test Stick (Liofilkem, Roseto degli Abruzzi, Italy), Aesculin activity was revealed by Aesculin Bile Test (Liofilkem, Roseto degli Abruzzi, Italy), and Voges-Proskauer test (Liofilkem, Roseto degli Abruzzi, Italy). Strains were then serotyped by slide agglutination with commercial antisera directed against the O antigen, O:1-O:3, O:5 and O:9 (Denka SeiKen, Tokyo, Japan).

DNA Microarray

Analysis of virulence features was performed by using V4.1 Microbial Diagnostic Microarray (MDM), the species-specific microarray slides were designed by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (Italy) and printed/spotted at the Microarray Laboratory at the NRC-BRI in Montreal, Canada, as described by Tonelli and colleagues (Tonelli *et al.* 2012). Out of 316 sequences for the genus *Yersinia* contained on this microarray, 228 sequences, designed in 2006 specific to *Yersinia enterocolitica*, have been reported in Annex 1, Supplementary Table I [Delihas 2003 and National Center for Biotechnology Information (NCBI) nucleotide sequence collection]. In this study the genes taken under consideration were the virulence factors and plasmid, amongst them there were genes for the T3SS secretion apparatus named *ysa, ysc, yop, lcr*; the T2SS secretion apparatus, named *yts1*, the virulence adherence type IV pili, and other chromosomal virulence genes, named *virF, yst, fla, rtxA*.

The specificity and sensitivity of the oligonucleotides present on the microarray in detecting virulence genes were tested by performing the analysis also with 2 reference strains: the strain ATCC 51871 O:8 1B, which is a highly pathogenic bio-serotype harbouring specific virulence genes, and the strain NCTC 11176 O:3 biotype 4, which is the bio-serotype most frequently detected in this study.

To perform microarray analysis, the strains were

Table I. *Number of pigs sampled for* Yersinia enterocolitica *isolation at a slaughterhouse located in Abruzzo Region, Central Italy, from February to April 2011.*

Farm	28 February	7 March	14 March	21 March	28 March	4 April	11 April	Number of pigs sampled
A	2						1	3
B	2	1	1	2	1	3	2	12
C	2			2				4
D	2							2
E	41	41	35	39		33	20	209
F	2		2		1	2		7
G	2	1	1					4
H	9							9
I			1	1	1	1		4
L				24	23	24		71
M				8			10	18
N					4			4
O					29			29
	62	**43**	**40**	**76**	**59**	**63**	**33**	**376**

grown overnight at 37 °C on Blood Agar, colonies were collected, and washed 3 times with ultrapure water.

Extraction and purification of strains DNA

For isolation of genomic DNA, the Maxwell® 16 Tissue DNA Purification Kit (Promega, Madison, Wisconsin, Italy) was used according to manufacturer's protocol. After extraction, DNA was purified using a DNA purification kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. An amount of DNA from 300 to 2,000 ng was labelled with Cy™3-dCTP (GE Healthcare, Buckinghamshire, UK) using BioPrime® Array CGH Genomic Labelling Module (Invitrogen, Carlsbad, CA, USA), as described in the provided protocol.

This mix was incubated at 37 °C for 2 hours in order to obtain the labelling reaction and finally stopped by adding 5 µl of stop buffer. Unincorporated deoxynucleotides were removed from the labelling reaction using QIAquick DNA purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Finally, 2 µg of the eluate containing purified and labelled DNA were dried under vacuum in a rotary dessicator (Savant SpeedVac®, Sunnyvale, California, USA).

Hybridization, image processing, and data analysis

The dried labelled DNA was suspended in 25 µl hybridization buffer consisting of 400 µl of Dig Ease Buffer (Roche Diagnostics spa, Milan, Italy), 20 µl of Bakers yeast tRNA (10 mg/ml) (Sigma Aldrich spa, Milan, Italy), and 20 µl of Salmon Sperm DNA (10 mg/ml) (Sigma Aldrich spa, Milan, Italy). This solution was heated for 5 minutes in a thermoblock at 95°C and then immediately cooled in ice for 5 minutes. Microarrays were hybridized overnight at 42°C in a Slide Booster (Advalytix, ABI, Milan, Italy). After hybridization, stringency washes were performed with Advawash (Advalytix, ABI, Milan, Italy) using 1XSSC, 0.02% SDS preheated to 42°C. Microarrays were then scanned on ScanArray® software (Perkin Elmer, Milan, Italy) with excitation at 540 nm and emission at 570 nm. Scanned images were uploaded as TIFF files and analysed by QuantArray vers. 3.0 software (Perkin Elmer, Boston, Massachuset, USA) for analysis of fluorescent intensity.

The local background value was subtracted from the intensity of each spot. Spot parameters including morphology of spots and background signals, positive control (eub 338335, 16S Ribosomal RNA gene), negative control (arabid70 e gpf 50, green fluorescent protein), and empty spots were considered, and poor spots were flagged for elimination from the analysis.

All subsequent data analyses were performed using Microsoft Excel®. The median values of all empty spots were subtracted from the median values of each probe (3 spots for a single probe). The $\log_2$ of the median of the signal was calculated for each probe. These hybridization data were clustered using various algorithms with Cluster 3 software (Version: 3.0, University of Tokyo) and visualised by Java 3 view (version: 1.1.6r2) to show the comparison of the hybridization profiles. Uncentered Pearson Correlation and Complete Linkage method were used for hierarchical clustering. A threshold was determined so that each value below -0.18 (green) was considered absent and those above 0.18 (red) were considered present. Values between -0.18 and 0.18 were regarded as ambiguous present/absent.

Table II. *Number of pigs found positive for* Yersinia enterocolitica *isolation at a slaughterhouse located in Abruzzo Region, Central Italy, from February to April 2011.*

Farm	28 February	7 March	14 March	21 March	28 March	4 April	11 April	Total
A	0/2						0/1	0/3
B	0/2	0/1	0/1	0/2	0/1	3/3	0/2	3/12
C	0/2			0/2				0/4
D	0/2							0/2
E	0/41	4/41	3/35	6/39		12/33	1/20	26/209
F	0/2		0/2		0/1	0/2		0/7
G	0/2	0/1	0/1					0/4
H	1/9							1/9
I			1/1	0/1	0/1	0/1		1/4
L				0/24	0/23	0/24		0/71
M				3/8			1/10	4/18
N					0/4			0/4
O					0/29			0/29
	1/62	**4/43**	**4/40**	**9/76**	**0/59**	**15/63**	**2/33**	**35/376**

Results

Isolation of *Yersinia enterocolitica*

A total of 35 out of 376 pigs' tonsils was found to be contaminated by *Y. enterocolitica* (9.31%; 95% CI 6.78%-12.67%), the same amount of positive samples was confirmed with both methods. From each positive sample, 1 or more typical colonies were selected. A total of 35 colonies, from ISO method, and 47 colonies, from direct plating, were selected for further testing. Isolates were obtained from samples taken from pigs coming from 5 farms out of the 13 tested, respectively from farms B, E, H, I, and M (Table II). All strains were analysed using the tests listed in ISO 10273:2003 until species attribution and confirmed by API 20E®. All isolates were identified as *Y. enterocolitica* (n = 35 + n = 47), but only colonies obtained from direct plating (n = 47) were used to perform pathogenicity tests and molecular characterization. This group was chosen because of the bigger number of colonies recovered from positive samples compared to the ISO method (Table III). The ISO method implied less selectivity for typical colonies with a higher presence of interfering flora contaminants while direct plating allowed a more rapid isolation.

Biotyping and serotyping

All 47 strains were oxidase negative, urease positive, hydrogen sulphide production negative, and positive for the fermentation of glucose. Nine out of 47 strains were lactose positive originating from pigs coming from farm E (overall 8 isolates in 3 sampling days) and farm M (1 isolate in 1 sampling day).

Out of the 47 strains isolated, the prevalent bio-serotype was 4/O:3, with 45 isolates out of 47 (95.74%, CI 85.75%-98.69%), followed by bio-serotype 4/O:9 (1 isolate, 2.13%, CI 0.51%-11.07%), and 3/O:9 (1 isolate, 2.13%, CI 0.51%-11.07%). In 1 sample (S006), 2 strains (ID013, ID029) were isolated, having the same serotype but different biotype, 3 and 4 respectively (Table III).

DNA microarray

Yersinia enterocolitica strains clustered into 2 groups (big and small groups), while the most pathogenic reference strain ATCC 51871 O:8 biotype 1B, clustered alone. Strains belonging to the bigger group – including NCTC 11176 0:3 biotype 4 strain – were characterised by the presence of genes for the flagellum transport mechanism, required for efficient invasion, and by the presence of plasmid virulence genes of the T3SS secretion apparatus genes, named Ysc.

A variety of type III secreted effector proteins, called Yops (*Yersinia* outer proteins) was also present in this group.

The smaller group of strains carried genes for the flagellum transport mechanism but not plasmid virulence genes (Figure 1).

Core components of the secretion mechanism T2SS system Yts1 present in ATCC 51871 O:8 biotype 1B were *yts1G, yts1H, yts1I, yts1J*, and *yts1L*. Some

Table III. *Identification of strains of* Yersinia enterocolitica *isolated from each positive sample originating from different farms by direct method.* Samples were collected at a slaughterhouse located in Abruzzo Region, Central Italy, from February to April 2011.

Farm	Positive samples (ID)	Strains isolated (ID)
E	S001	ID001, ID004
E	S002	ID003, ID019
E	S003	ID002, ID005
E	S004	ID012, ID028
E	S005	ID010, ID030
E	S006	ID013, ID029
E	S007	ID007, ID009
E	S008	ID006
E	S009	ID020
E	S010	ID014
E	S011	ID31
E	S012	ID32
E	S013	ID008, ID011
E	S014	ID016
E	S015	ID023
E	S016	ID033
E	S017	ID034
E	S018	ID021
E	S019	ID026
E	S020	ID018
E	S021	ID024
E	S022	ID017
E	S023	ID015, ID027
E	S024	ID025
E	S025	ID022
E	S026	ID035
M	S027	ID041, ID042
M	S028	ID037, ID038
M	S029	ID039
M	S030	ID040
B	S031	ID045
B	S032	ID046
B	S033	ID047
H	S034	ID036
I	S035	ID043, ID044

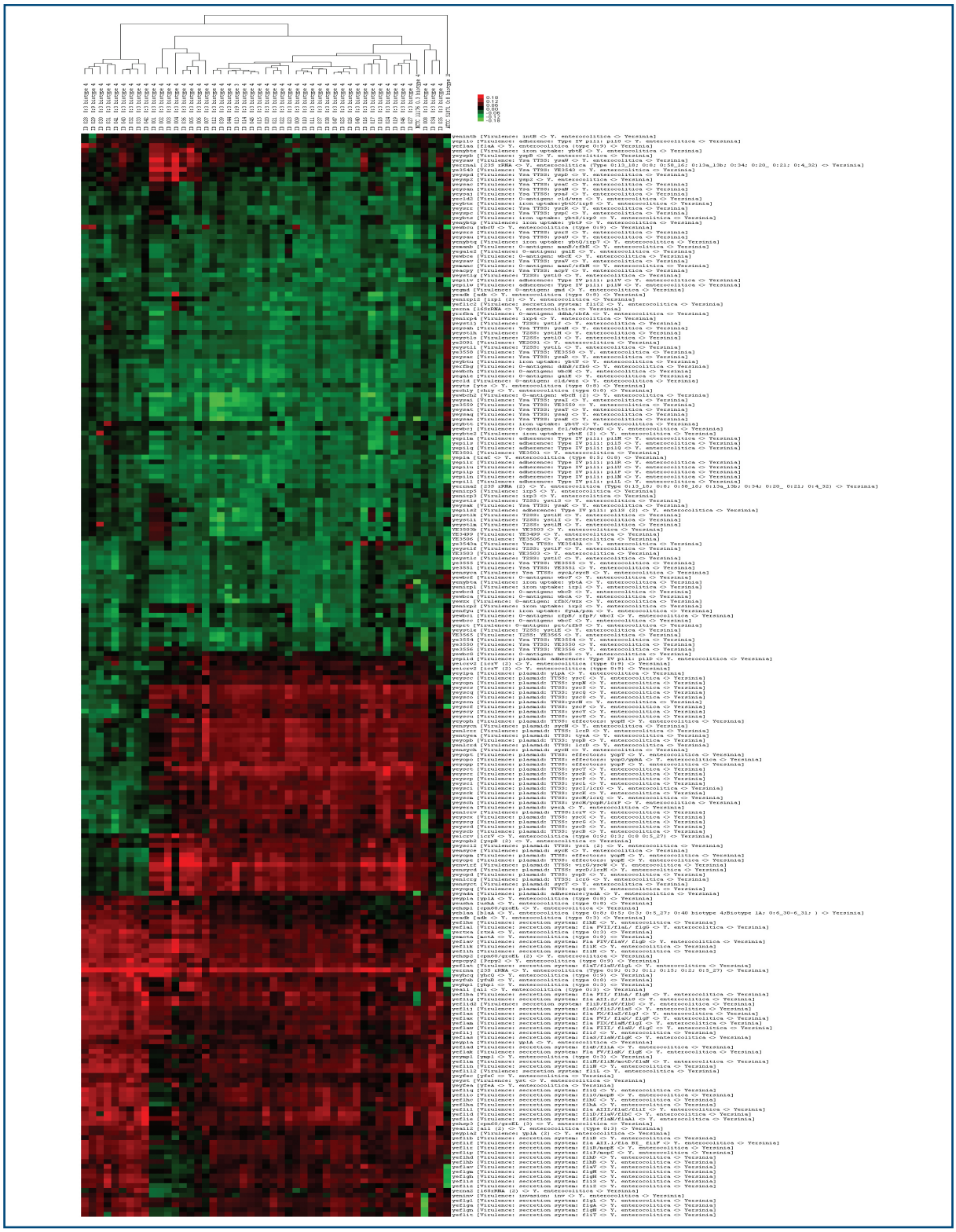

Figure 1. *The heat-map showing positivity/negativity for sequences to genes of* Yersinia enterocolitica. Each row corresponds to a gene and each column to a strain of *Yersinia enterocolitica*. The presence or absence of gene is indicated with red or black and green colours, respectively. The name of each gene is indicated at right. ATCC 51871 0:8 biotype 1B gave positive hybridization for most of genes for the flagellum transport mechanism, for plasmid virulence genes of the T3SS secretion apparatus genes, and for some *yts1* genes like *yts1G*, *yts1H*, *yts1I*, *yts1J* and *yts1L*. The hybridization profiles of ATCC 51871 0:8 biotype 1B are distinct and different. NCTC 11176 0:3 biotype 4 was positive for most of genes for the flagellum transport mechanism, for plasmid virulence genes of the T3SS secretion apparatus genes but not for core component *yts1* genes and *ysa. Yersinia enterocolitica* strains analysed clustered into 2 groups. Strains belonging to the bigger group were characterized by the presence of genes for the flagellum transport mechanism as well as by the presence of plasmid virulence genes of the T3SS secretion apparatus genes. A variety of type III secreted effector proteins, called Yops (*Yersinia* outer proteins), was also present in this group. The smaller group of strains carried genes for the flagellum transport mechanism but not plasmid virulence genes.

probes belonging to the *yts1* of T2SS were absent in most of the analysed strains, except of the ID001, ID002, ID003, ID017, ID018, ID019, ID024, ID030, and ID031 strains. The virulence genes *ysa* of T3SS *ysaC, ysaE, ysaH, ysaI, ysaN, ysaQ, ysaR, ysaU, ysaV ysaW* were present in biotype 1B O:8 and in a very few of the isolated strains. Specifically the *ysaW* probe produced a higher fluorescent response to the hybridization in the isolate strain ID004 and a lower florescence in ID001, ID002, ID005, ID016, ID016, and ID035. The *virF* gene was present in most of the strains belonging to the bigger group.

The *rtxA* gene was present in all the analysed strains, with the exception of the ATCC 51871 O:8 biotype 1B. The Type IV pili system was absent in most of the strains except for *pilO* that was present in 1/3 of the strains. Other chromosomal virulence genes like attachment and invasion locus (*ail*) were found in most of all isolates, but not in ID004 strain. Invasin gene (*inv*) was absent in more than a half of the analysed strains. Enterotoxin gene (*yst)* was found prevalently in the smaller group (Figure 1).

Discussion

The prevalence of *Y. enterocolitica* recorded in this study (9.31%; 95%CI CI 6.78%-12.67%) was lower than the one reported during previous years from research performed in Northern Italy abattoirs. In particular, a study carried out in Italy from 2005 to 2007 on 428 fattening pigs reported a prevalence of 32% (Martínez *et al.* 2011); Bonardi and colleagues in a study from 2006 to 2007 on 125 samples reported a prevalence of 15.2% (Bonardi *et al.* 2010). A study published in 2013 (Bonardi *et al.* 2013) reported a prevalence of 10.8% in samples collected from 2005-2008. A more recent study, conducted in 2012, showed a prevalence of 15.3% (Bonardi *et al.* 2014).

The direct plating method allows for a more rapid isolation of the pathogen and it is an easier and less expensive method, enabling the selection of single colonies and reduced polymicrobial flora, as reported by other authors (Van Damme *et al.* 2010). However, this method was not able to increase the recovery of positive sample compared to the ISO method that implies 2 isolation steps and causes loss of selectivity and a higher presence of not-target flora.

Biotypes 2-5 are generally considered less pathogenic compared to biotype 1B strains, while biotype 1A strains are classified as non-pathogenic (McNally *et al.* 2004). The predominant biotypes may vary according to geographical region, with biotype 1B strains predominantly isolated in North America and from sporadic cases in Poland (Bottone 1999, Gierczynski 2009), while biotype 4 strains (serotype O:3) are predominantly isolated in Europe, Japan, Canada, and USA (Fredriksson-Ahomaa *et al.* 2011, Gierczyński *et al.* 2009, Poljak *et al.* 2010, Paixão *et al.* 2013).

In this study the positive samples, coming from 5 farms out of the 13 tested, were bio-serotype 4/O:3, followed by 3/O:9, and 4/O:9. These bio-serotypes are all pathogenic for humans, demonstrating that pig is a natural reservoir of *Y. enterocolitica* in Italy. Nine out of 47 lactose positive colonies were obtained in pigs coming from 2 farms only.

To date, 6 secretion systems (T1SS-T6SS) have been characterised in Gram-negative bacteria and the process of secretion is crucial to manipulate their environment and exploit whatever nutrient sources are present. Highly complex mechanisms are involved in these systems (Francetic *et al.* 2000). The Yts1 T2SS seems to be present in all of the highly pathogenic *Y. enterocolitica* serotypes (serotype O:8, O:13, O:20, O:21), but not in the low-pathogenic *Y. enterocolitica* isolates (e.g. O.3, O:9) (Von Tils *et al.* 2012). Results from microarray analysis showed how strains cluster. The virulence gene patterns obtained from isolated strains were almost similar among all the tested samples within the 2 obtained groups. As a matter of fact, the isolated strains were not found to be different at farm level, although the strains belonging to the bigger group could be more virulent than those belonging to the smaller one, which lacks the virulence plasmid.

ATCC 51871 Biotype 1B O:8 strain and strangely few low pathogenic strains isolated during the trial were found positive for some genes of the secretion mechanism Yts1 T2SS, and the virulence genes T3SS named *ysa* (*Yersinia* secretion apparatus). These genes are only prominent in the chromosome of highly pathogenic *Yersinia* species and not in low pathogenic strains. The results of the microarray assays must be confirmed by further molecular studies with more specific techniques in order to confirm the gene presence because the positive signal could be aspecific. However, it is well known that *Y. enterocolitica* or other *Enterobacteriaceae* have the capacity to acquire and/or loose genes that can increase the ability to infect humans and/or enhancing the function of proteins responsible for virulence (McNally *et al.* 2016).

The chromosomal virulence genes, like attachment and invasion locus (*ail*) and invasin gene (*inv*), which encodes invasion, a protein functioning in the invasion, and adhesion to the host cell, were found in half of isolates. The *inv* gene can be present in pathogenic and non pathogenic strains, but in the last ones it is not functional (Schneeberger *et al.* 2015), whereas recent studies showed that *ail* gene can be also present in non-pathogenic strains 1A and, for this reason, their pathogenicity has previously been addressed (Paixão *et al.* 2013b).

All strains were characterised for the presence of genes for the flagella secretion system. This last apparatus appears to be required to ensure the bacterium migration and contacts to the host cell. Non-motile strains of *Y. enterocolitica* were less invasive than motile strains (Young *et al.* 2000). At the same time, many strains were positive to *ysc* and *yops* genes, important for bacteria manipulating function on the host immune system.

This preliminary study based on the use of a DNA microarray for the characterization of *Yersinia enterocolitica* strains should be carried out on other isolates to be properly evaluated and validated. According to the results obtained in this study, pigs in slaughterhouses may be a source of infection for humans. Therefore preventive measures like hygienic handling, dehairing, and evisceration of pork should be adopted during the process to decrease the potential transmission of this pathogen.

To assess the relevance of *Y. enterocolitica* for public health, an official monitoring program should be implemented on foodstuffs at risk, taking into account that consumption of raw or undercooked pork products is a common habit, especially in some Italian regions.

To date, no systematic collection of data on human infections with *Y. enterocolitica* is in place. Moreover, no surveillance plan is in place for the detection of this bacterium in products of animal and/or vegetable origin that could be related to transmission to humans. No methods for a rapid detection of *Y. enterocolitica* in clinical and/or food samples have yet been developed. As far as the latter are concerned, it has been clearly identified that there is a need for procedures with higher efficacy for direct isolation than the one described in the ISO 10273:2003. An implementation of a national surveillance plan is advisable, in order to assess the impact for this pathogen in the Italian pig production as well as to identify the corrective actions that should be taken.

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Annex 1

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Gene Name	Name of sequence spotted on microarray	Gene product	Oligonucleotide probes (5'-3')
16S rRNA	*yerna2*	16S ribosomal RNA	acc gca taa cgt ctt cgg acc aaa gtg ggg gac ct
16SrRNA	*yerna*	16S ribosomal RNA	ata aag gtt aat aac ctt tgt gat tga cgt tac tc
23S rRNA	*yerrna1*	23S ribosomal RNA	cac gga agt gga taa tga ttg acg gag cgc agc gac gtc aat gcg tcc aat aaa gtc gag ttg gct tag g
23S rRNA	*yerrna*	23S ribosomal RNA	cgt gac gca aag ccg gca tgt tca agc cac act aaa cgt tga gtt ggc cgg tgt gct gac aaa cga aca g
23S rRNA	*yerrna2*	23S ribosomal RNA	gtg gat aat gat tga cgg agc gca gcg acg tca atg cgt cca ata aag tcg agt tgg ctt agg gat acg t
acpY	*yeacpy*	serine rich protein / acyl carrier activity	atg cct aat atc atg gca ccc agt act gtt act act ccc aga cct tta aat tct gct tct ccc aag ttt t
adK	*yeadk*	adenylate kinase	ggt cgt gag ccg tgg ttg acc cca tat acc gac gaa acc tta aaa agt ctg ccc tct caa ggt gtt aaa c
adk	*yeadk*	adenylate kinase	cat ttg aga act ctg agc ctg tta tta tac gga atc cat atg cta tac gtc ctt ggc agc atg ttt tag a
ail	*yeail*	attachment invasion locus	gct gct cac gga aag gtt aag tca tct gta ttt gat ggg tca gtc agt aca agt aag acg tca atg gca t
ail	*yeail2*	attachment invasion locus	tgg tta tgc aca aag cca tgt aaa aga aaa tgg gta tac att gga taa tga ccc taa agg ttt taa cct g
blaA	*yeblaa*	beta-lactamase A	aaa acg ggt agt ggc gat tac gga acc acc aat gat att gcg gta ctt tg
chly	*yechly*	Chiy protein	tct gtg taa ttc cag gtg gcc cgg ctt cga cga cag gtt caa cca ccc aca ctt gta ata ttc ctg agc g
cld	*yecld2*	O-antigen chain length determinant	caa cag gtt gag aat gaa cat aaa atc aga atc cag aga tta gaa tac gca gct tcg ata gct aaa gcg g
cld	*yecld*	O-antigen chain length determinant	ggt ttg cag aag cca gct att ggt gca ttt gat att tct gct agt agt aat aat tat cca ata tcc atg g
flaA	*yeflaa*	putative flagellin FlaA	aaa aga aaa aca gca taa gct gga gca gca att aaa aac aca gca agc ggc act gga taa aca aaa atc g
flaD	*yeflad*	RNA polymerase sigma factor for flagellar operon	tgc tga atg ctg ttg agc gtt atg acg ctt tac aag gaa ccg cat tta cca ctt atg cgg tac agc gta t
flaK	*yeflak*	flagellar hook protein FlgE	act tta acg atg gta cca cca cca cca cta acc gcc gtc tgg atc tgg cta tta gtc aaa gtg gtt tct t
flaL	*yeflal*	flagellar basal-body rod protein FlgG	aca cca aag atg tgg cta tca aag gcg agg ggt tct ttc agg tac aaa tgc ctg atg gca cca atg cct a
flaM	*yeflam*	flagellar P-ring protein precursor	ctg agt aac atg ttg tcc caa ctg ggg att acc gtg cca ccg ggc acc aat atg cag ctc aaa aac gtg g
flaS	*yeflas*	flagellar hook-associated protein	ata acg cct tta tta cca acc aat tgc gtg cct cgc aaa cgc aaa gca gtg ggc tga caa cct att atc a
flaT	*yeflat*	flagellar hook-associated protein 3	aaa cgg gtg gtc aat cct tct gat gat ccg atg gcg gcc tct cag gcg gtt atg gtg tca cag tcc cag t
flaV	*yeflav*	basal-body rod modification protein FlgD	ata aca gcc aat ctt tgc agg cga gca gcc tga ttg gtc gcg gtg tga tgg ttc cgg gaa cca acg tgt t
flaV	*yeflav*	flagellar hook-associated protein 2	gta ttt tgc aaa gtg cgc tgg caa aag tgg aaa ccg ctt cag ccg cgc tga aaa aag ctg aca cac tga c
flaW	*yeflaw*	flagellar basal-body rod protein FlgC	ccg gca atc cgc tgg ctg atg cca agg ggt atg ttc gta tgc caa acg ttg atg tga ccg gcg aga tgg t
flaX	*yeflax*	flagellar basal-body rod protein FlgF	ccc gat ggc act gag gct tat acc cgt aac ggg aat att caa atc tcg gct gat ggg caa atg aca gtg c
flaZ	*yeflaz*	flagellar protein FlgJ	cgc tgc cgc aag atg gcg tta tga aca gtg atc aaa cgc ggc tct ata cct caa tgt atg acc aac aga t
flbA	*yeflba*	flagellar basal-body rod protein FlgB	atc ccg gca agt aca gtt cag cca cct gat ctt gat ttg tta ttc cgt gtc ccg gat cag cca tca atg g
flgA	*yeflga*	flagella basal body P-ring formation protein FlgA	cag acc caa gta caa gtc agt ggc cat tat gca gtg gcg gct cgt caa ctg gca gcc ggt gca aaa atg a
flgH	*yeflgh*	flagellar L-ring protein precursor	ttg aga tgg gtg ggg gcg cct ttg atg gca acg ctg cta ctc aat ggc tgc gcg tat att ccg cac aaa t
flgL	*yeflgl*	flagellar hook-associated protein 3	agc aaa ata tgc agg gtg tca cta atg ccc aat ccc tgt gga tgc agt cgg gcc aac aac tct cga cgg g
flgM	*yeflgm*	negative regulator of flagellin synthesis	tag aac gtg tcg aaa cct taa aac agg cga tcc gtt cag gcc aac tga cca tgg aca ccg gta aaa ttg c
flgN	*yeflgn*	flagella synthesis protein FlgN	gtt caa ttt tgg cga cct tgg ctt atc tgg acc aga ccc ggc tca cca ccg aaa aaa aca taa ata ttc a
flhA	*yeflha*	flagellar biosynthesis protein FlhA	ttg ctg gtt gcg atg ttt act cag cgt act ttg gat ttt gct gca ttc ccg acc att ctg ttg ttc tcg a
flhB	*yeflhb*	4 probable transmembrane helices predicted for YE2567 by TMHMM2.0	ggg gga atc gat ggc gcg tca act ggc tgc cat gat tgc tca ggg gtt gca ttt tga tca cgg cct tat c
flhC	*yeflhc*	flagellum biosynthesis transcription activator	ggc agc cct cca cct aaa gga atg tta ccg ttt tcg acc gat tgg ttt atg act tgg gaa cag aat att c
flhD	*yeflhd*	flagellar transcriptional activator	atg act tgc agc aaa tcc aca ccg gaa ttt tat tgt cga gtc att tac tcc acg agc tat cgt taa aag a
flhE	*yeflhe*	flagellar protein FlhE precursor	agc cgc tgc aat cac cgg tcg cac tgg cgc aag ata atg cgc gaa ttg tat cga taa gtt ggc gct acc a
fliB	*yeflib*	flagellin lysine-N-methylase	caa ttg ccc tta ttt gga tca aga tca act gtg caa agt aca taa gcg ttt agg ggc gga tgc ttt aag t
fliC	*yeflic2*	flagellin	aac tgc ttg gga tac tgc caa gac ctt agt aac gac caa tac tgg ttt ata caa tac agc act gaa aac c
fliD	*yeflid*	flagellar hook-associated protein 2	atg gca agt atc agt tcg ctg ggc gtt gga tca ggc atg gag tta agt tct ctt ttg acg agt tta tca a
fliD	*yeflid2*	flagellar hook-associated protein 2	tgc aga aca agc gcg att aac acc gct gac cac aca gca aac cag tta taa aag caa act tac cgc tta t
fliE	*yeflie*	flagellar hook-basal body complex protein FliE	agc aaa cag ccc gta cgt tag cgc aga att ttg aat tag gtg tgc ccg gcg ttg gca taa atg acg tga t
fliF	*yeflif*	flagellar M-ring protein	cta aaa gcc cag att atc gcg ttc ttt ata gca acc tga atg atc gtg atg gtg gtg ata tcg tca cgc a
fliG	*yeflig*	flagellar motor-switch protein	aac agt tgg ttg atg tat tgg ctg agt ttg aag acg atg cag agc aat atg ccg cac tga gtg tga atg c
fliH	*yeflih*	flagellar assembly protein	ggt gat aaa cag cag ata ttg atg aac ctg caa ctc gaa gcc gag aag cag ggt cgc cag caa gga ttt g
fliJ	*yeflij*	flagellar protein FliJ	aat acc ggg tgc ggc tga atg aca cac tga gta acg gca tgg cct ctt cca gtt ggc aaa att atc agc a
fliJ	*yeflij*	flagellar protein FliJ	atg aaa aat cag tca cct ctc gtc acc ctg cgc gac ctg gct caa aag gcg gtc gag cag gca agt act c
fliK	yeflik	flagellar hook-length control protein FliK	cac gct acc tgc ggg ttt gac tca tgc cgg aca gac tgg cga agc agc cgg tag taa gaa aac cgc aat c

continued

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Gene Name	Name of sequence spotted on microarray	Gene product	Oligonucleotide probes (5'-3')
fliL	*yeflil*	flagellum-specific ATP synthase	ctg aaa ccg gct acc gcg ccc cgc tta tca ctc ccc cga tca acc cat tac aac gta ccg cga ttg aac a
fliL	*yeflil2*	flagellar protein FliL	tac cgg tga tcc cgg tgt tta tgc cac tgg ata cct tca cgg tca atc tga tca cac ccg ata ata atc t
fliM	*yeflim*	flagellar motor switch protein FliN	agc gcc tcc atg cgc tgg aga taa tca atg agc ggt ttg ccc gtc agt tcc gta tgg ggc tat tta acc t
fliN	*yeflin*	flagellar motor switch protein FliN	gcg cct gac gca ctg ggt aat cta caa gat atc gat ttg att ctt gat atc ccg gtg aaa ctg acc gtt g
fliO	*yeflio*	flagellar protein FliO	gtt gtc agg ttg gtc agc gcg aac gcg ttg tta ttg tcg agg tgg aca aca ctt ggc tgg tat tgg gtg t
fliP	*yeflip*	flagellar biosynthetic protein FliP	tca tca tgt cgc cgg tgt ttg aca agg tct atc aag atg ctt atc tgc cct tca gcc agg aca aaa taa g
fliQ	*yefliq*	flagellar biosynthetic protein FliQ	cca ctt tag tta ttg ctg gcc cgt gga tgc tca gcc tga tcc tcg act ata tgc gta att tgt tca cca g
fliR	*yeflir*	flagellar biosynthetic protein FliR	tat taa tta cct ttc tga ttg ctc cat ccc tgc cac cgg taa ata ttc ctt tag tct cca gcg gag ctt t
fliS	*yeflis*	flagellar protein FliS	tta ata atg ggc tga gtg cag gcc tta ata tgg aaa aag gcg gtg agc tgg cag aga att tgt cgg ctt t
fliT	*yeflit*	flagella protein	cta ata tca cta ttt ctt cat gct cat cgc taa tgc tgc aag att tat tac ggg aga agc taa gag cta t
fliZ	*yefliz*	putative alternative sigma factor regulatory protein	ttg atg atc aag tct ggt caa aac ttc agc atg aat taa ccg cat tgt gtc gtt ttt gca gtg aga tat a
fyuA	*yenfyu*	Fur protein	cgt tca tgt agc cat agg tac ggt aac gac ggt cga aca ggt tat cga cat gga cgg aaa tgt tta tcc g
galE	*yegale*	UDP-glucose 4-epimerase	agc taa tgt caa aaa att aat att cag ctc ttc agc aac agt ata tgg tga gcc tga att cgt tcc gtt g
galE	*yegale2*	UDP-glucose 4-epimerase	ggt gct gga tat ata ggt tct cat aca gtg ctt aca ttg ttg gaa caa gga agg aat gtt gtt gtt ctt g
gmd	*yegmd*	GDP-mannose 4,6-dehydratase	cct ggt aaa aga aat tca acc tga cga aat cta taa ctt agg tgc tca atc tca tgt ggc tgt ttc att t
hsp	*yehsp1*	heat shock protein	ctc aga ctc aac tgt ggg tga gct gat tgc aca agc gat gga aaa agt cg
hsp	*yehsp2*	heat shock protein	ttg ctc agg tag gga cca tct ctg caa act cag act caa ctg tgg gtg agc tga ttg cac aag cga tgg a
hsp	*yehsp3*	heat shock protein	acg cgg tat cga caa agc agt tat cgc tgc ggt tga aga gct gaa aaa act gta tgt acc atg ctc tga t
intB	*yenintb*	P4-like integrase	ccg acc acc act gca tca tcg ctt tgc ggg ctt cta ggt gct ctg cct tat gaa tat aag cca tgc gca c
invA	*yeninv*	invasin	att tta att cag aag cgt tag aga acc ccg ctg aac ata atg agg ctt tca ata aga taa tca gta ccg g
irp1	*yenirp1*	synthesis of siderophore yersiniabactin	ccc ggc gta tgc tca gtc agt aaa cgc agc aga tgc gcg atg ttg tcg agc cac gtt tct gcc gct tgc a
irp1	*yenirp12*	synthesis of siderophore yersiniabactin	gct tct ctt ctg cgc cga cag cag att cca ttg atg cat cga tcg ctc aac act acc cgg act gcg aac c
irp2	*yenirp2*	Yersiniabactin biosynthetic protein	atg att tct ggc gca cca tct aag gat tcg ctg tta ccg gac aac cgc cac gcg gct gat tac caa caa t
irp3	*yenirp3*	part of the siderophore biosynthetic operon	gcg tac acg tta ttc agg agc atc ccc tcc acc cgg atg aca tca gtt cct tac aaa cgc tgg cgc agg a
irp4	*yenirp4*	yersiniabactin biosynthesis	atc gcg atg atg ccg act tta tcg ctg aac tga tag aca ttg gcg gat gtt ctc ctg aac tgc ggg aaa a
irp5	*yenirp5*	part of the siderophore biosynthetic operon	gtt gac gaa ctt gcc gca ggt ctg tca tca ctg ggt ttg cgt tcg ggg gag cat gta att gtg cag ct
lcrD	*yenlcrd*	low calcium response locus membrane protein d (plasmid)	gcg tgt tgg aca ttc tga ttg ctg tta aca tga cca ttt cag tgg tgt tgt taa tga tag cga tct ata t
lcrG	*yenlcrg*	low calcium response locus protein g (plasmid)	tca ccg cgc aaa att att gca aga aat gtg tgc tga tat cgg ctt aac gcc tga agc cgt aat gaa gat a
lcrG	*yeyopd*	low calcium response locus protein g (plasmid)	tca ccg cgc aaa att att gca aga aat gtg tgc tga tat cgg ctt aac gcc tga agc cgt aat gaa gat a
lcrR	*yenlcrr*	*Yersinia enterocolitica* low calcium response locus protein R which may play an important role in its regulation	atg atg gaa gac cct tta att ccg tgg ctt acc gaa cat ggc ttg gtt tgc cac cct cat act tta tct g
lcrV	*yenlcrv*	V antigen (plasmid)	tac tgg tca tgg ttc ttc agt ttt aga aga att ggt tca gtt agt caa aga taa aaa tat aga tat ttc c
lcrV	*yeicrv*	V antigen	atg att aga gcc tac gaa caa aac ccc caa cat ttt att ggg gat cta gaa aaa gtt agg gtg gac caa c
lcrV	*yeicrv2*	V antigen (2)	ata tga tcc cca aaa aga ttc gga agt ttt tgc cga tag ggt aat tac tga tga tat cga att act caa g
lcrV	*yeicrv2*	V antigen (2)	ata tga tcc cca aaa aga ttc gga agt ttt tgc cga tag ggt aat tac tga tga tat cga att act caa g
manB	*yemanb*	phosphomannomutase	aaa att agc act gtc gcg tgg ttt aca aga tgc cgg tgt tga tgt att aga tat tgg ttt atc tgg tac g
manC	*yemanc*	mannose-1-phosphate guanylyltransferase	ccc atc ttt cac cat tag tga tat gta atg cag aac ata ggt tta tta ttg ctg agc aac tta ggc agc a
motA	*yemota*	flagellar motor protein MotA	atg cag aaa tta ata ggc ctg ctg gtc att atg gca tgt gtg att ggc ggc tat ata atg tca ggc gga a
pcpY	*yepcpy2*	Pcpy2 protein	gca ata gtg act cgc tgg ccc ggt tgg aac tgc gtt acc cct tga gcc tga acc acc agg aag gtg gtg c
pilD	*yepild*	type IV prepilin peptidase PilD	tgc tct cgc taa gag tat cca tag cat tgc ggt tgg tgc tag tgc tga agc agc gaa aca agc tgc agt t
pilL	*yepill*	putative type IV pilus operon lipoprotein	caa gtt ggt atg gct cac cct atc act att tgc cgc tgg ttg tac cca taa tac cac cgc tca aaa cga a
pilM	*yepilm*	putative type IV pilus operon exported poprotein	tcc gat aac cgt atc aac gct gcc ata cga cat gga cgt ttg tgg ttg tgg tta cct gaa cag caa ggg t
pilN	*yepiln*	putative type IV pilus operon lipoprotein	atg cat cgg ttt ttc gcc acc cga act tct cgc atc att agt gtg gtt ctc agt cta acg ttt ttg gct g
pilO	*yepilo*	putative type IV pilus protein	tgc tgc ctc aac aga taa tga aaa ttg gca gca tgg cgt ggg tgg cgg gta tga gct ggc agg tgc acg a
pilP	*yepilp*	putative type IV pilus protein	cct gtt ggc tat ggt tgc ctc tgg tta tgt ggg gcg gtt ccg tca atg cgg cag act ttc aga cct cga t
pilQ	*yepilq*	putative type IV pilus protein	tct ttg ttg agt atc tgc ctt gtg ggc aag tag tgc tgg tta ttg ata aca atc gac gca ccg atc cac g
pilR	*yepilr*	type IV pilus integral membrane protein	cgt ttt cgg cct gtc cgc aac atg tcg ttg gga cag cgc ctg cgc tat gaa ctg gta cgc cat acg ttt a

continued

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Gene Name	Name of sequence spotted on microarray	Gene product	Oligonucleotide probes (5'-3')
pilS	*yepils*	putative type IV prepilin	ggg ggg ctt tat atg ctg tgg aat cga aag gat att gcc ttg gag tca gcc aac gtg cag agc atc atc a
pilS	*yepils2*	putative type IV prepilin	act ggt acg gct acc cta tgg aat aca tgg ggt ggg cag gta gtg gtg gcc cct ata act gcg aat ggt t
pilU	*yepilu*	type IV prepilin peptidase	gtt acc ttg gtt gtt tgt gct gcc cct ttt tgg gct ggc act gtg gct act tga ccc aat aac ccg gca a
pilV	*yepilv*	putative type IV pilus protein	tga gca ctg gca ttg ctc tgt tga ttc tgg tta ttg tcg tga ttt ggg caa cgt cta ttt acg gtg act a
pilW	*yepilw*	putative transposase	aaa aaa tct gtg act taa aca ctc tac agt tgg aat cca gta gtt tta tcg aga atg acc tgc gcc cct a
pla	*yepla*	type IV secretory pathway VirB4 component	tca aaa atg cac gtc ctc gct atg gtg agg tct tca ttg ata gtc ctt tag gaa tgg gcg tag cgc gtc t
prt	*yeprt*	paratose synthase	caa tag aaa att tac tca gaa gta ata ttg aat tcc cta cga aac tac ttg aag cca tgg agg ttg cag g
rfbA	*yrrfba*	glucose-1-phosphate cytidylyltransferase	ttc atg cat atg tct gac att act ttc tct atg cgt gat aat gaa atg aaa gta cat cag aag cgt gtg g
rfbG	*yerfbg*	CDP-glucose 4,6-dehydratase	tta ttt gaa gca gcc aac gtt gcg gat ggt atg att tcg gaa gta ggt gat att cgt aat tat gct cag c
rtxA	*yertxa*	putative cytotoxin RtxA	gtg atg ata cga taa aag gag gag cag cat atc tgg agg ttt tag ata cta ctg gga agt taa ccg tga a
sycA	*yensyca*	similar to *Salmonella typhimurium* SicA	acc aga aac atg atg cgg cag aaa atc aaa cgg aag ata tgg ata tta ttt ttg atg aac ttt gtc agg c
sycD	*yensycd*	yopB/D chaperone/regulatory protein SycD	atg caa caa gag acg aca gac act caa gaa tac cag ctg gca atg gaa tcc ttc cta aaa gga ggg gga a
sycE	*yensyce*	SycE, YerA; putative YopE chaperone (Tir chaperone family protein)	caa gct atc act caa tta ttt caa caa ctt tcg ttg tct att cca gat act att gaa ccg gtt atc ggt g
sycH	*yensych*	YopH chaperone SycH (plasmid)	gcg cac tta cag ttc att act tga aga att tgc tac aga gct agg tct tga aga aat aga aac aaa tga g
sycN	*yensycn*	yopN chaperone SycN (plasmid)	gcc tct tat tca att aga gat ggc tca atc tgg cac gct gca act gga aca aca tgg tgc gac act gac a
sycT	*yensyct*	yopT chaperone SycT	atg cag aca acc ttc aca gaa ctt atg caa cag ctt ttc ctg aag ctt ggc ttg aac cat caa gtt aat g
tyeA	*yentyea*	control of yop release; required for delivery of yopE and yopH (plasmid)	aac atc ttg cca acg cct ttt ccc ttc cta cgc ctg aaa tca aag tgc gtt tct atc aag att taa aaa g
ushA	*yeusha*	putative UDP-sugar hydrolase	atg ata ttg gta tgt gtc acc cca ttt tca ctc att aaa ctg gcg ctg aac ggc ctg gtt tta acg aca a
virF	*yenvirf*	virulence regulon transcriptional activator (plasmid)	gcg tat tgt ggg gga ggt gcg tat gtc acg acc att atc tcg cac agc aca cat tga tgt tag tat att t
virG	*yenvirg*	Signal peptide predicted for YEP.37.(Putative type III secretion effector targeting lipoprotein [Precursor])	gcg tat tgt ggg gga ggt gcg tat gtc acg acc att atc tcg cac agc aca cat tga tgt tag tat att t
wbcA	*yewbca*	putative epimerase	ggc cag ttt atg agt ttc ata cta aat ccg cat aac aga cgg agt ata ttt tta gca aaa gga ata gct c
wbcC	*yewbcc*	putative glycosyltransferase	cat tgt aaa gag ggc tag atg gga agc cat aga att tga tga taa tta tat tgg gag tgc ata tcc aca c
wbcD	*yewbcd*	putative 6-deoxy-D-Gul transferase	tca atc ttt tag ctg gtt cgt cgc atg cat att gtt gtt tct ggc taa ata tgc tta aca gag aat gtt g
wbcE	*yewbce*	putative membrane protein	tta aat tat aac tta aaa ata tcg aag tcg atg tgg ctt ctc ctc tgc ctc ata gcc ttt gta cgt ttc g
wbcF	*yewbcf*	hypothetical protein (plasmid)	aaa act ata atc ttt cat ggc tca gcc agc aaa aat tgg ttt cag aag gct aaa gaa cta tat gtc gga g
wbcG	*yewbcG*	putative glycosyltransferase	aga tat tcg atg taa atg taa tgt ttg aga tat act gtt aga tgt taa tct ata gga aag taa atc ctc a
wbcH	*yewbch*	putative glycosyl transferase	tca cta tgg ata ttt tca att tcc aga tgc tgc gct ata ttc ttc gga ttt ttt gag agt tag cat agc g
wbcH	*yewbch2*	putative glycosyl transferase	ccc gac taa tca ctt cga tat aat atc atc att acc ttt tat taa ccc tct ttg tca tcc tgc aac cat g
wbcI	*yewbci*	putative galactosyltransferase	atc gag cta atc aag cat tta aag ttg gct ggt gta gac ttt act ctt aat gtt gtt ggt gat act tac c
wbcJ	*yewbcj*	GDP-fucose synthetase	agg caa ttg atg agg ttt acc ttg cag cgg cga aag tag gag gaa tcc agg cca ata aca att atc ccg c
wbcU	*yewbcu*	O-antigen biosynthesis	tag aaa ata aaa gtt tta tta ttg ctg ggt acg gat tac ctg cgg aaa tag gga tga cag ttc ttt ttg g
wzx	*yewzx*	previously sequenced as *Yersinia enterocolitica* putative O-unit flippase Wzx or RfbX SWALL	cat ttt tcc aaa cta tca gta ttc ttg ggt tgg act caa ttg ttg ttc gag atc tat cca atg gcg tag a
yadA	*yeyada*	putative YadA invasin	tgc tct cgc taa gag tat cca tag cat tgc ggt tgg tgc tag tgc tga agc agc gaa aca agc tgc agt t
ybtA	*yenybta*	transcriptional regulator ybtA	tca tga cgg agg tat tgc cgt tag cca ggc gtc ttc tgg ctt cct gca ttc gtt cag cct gaa aca ggc c
ybtE	*yenybte*	Yersiniabactin siderophore biosynthetic protein	cgg tga gcg gcc agt ggt cca gaa act cga ttt ggt cag gaa ttt tcc acg cgc tga gcc cca tac ggg t
ybtE	*yeybte2*	Yersiniabactin siderophore biosynthetic protein	atg aat tct tcc ttt gaa tct ctg att gaa cag tat ccc tta gcc att gcc gaa cag ttg cgc cac tgg g
ybtP	*yenybtp*	lipoprotein inner membrane ABC-transporter	att aac cag cct ggg ggc gct cct ttt tct ggc gtg gag cct gcg cga cat tcg cgc gac gcc tga cgc t
ybtQ	*yenybtq*	inner membrane ABC-transporter ybtQ	gcg gca ttc atg cag ggg atc gcc ttt gcc tgt ctt tat ccg atc att gat gcg ctg tta cgg gga gac g
ybtS	*yeybts*	putative salicylate synthetase	acg cca gtt cgc aga aga aga gtg tta tgt cta tga gcg tca acc ctg ttg gta ttt agg caa agg gtg c
ybtT	*yeybtt*	Yersiniabactin biosynthetic protein ybtT	caa tct gcg taa aaa agg acc ggg ctt gtt gaa tgg gat aga aat gat cgc cgt caa tca cca ccg ggc c
ybtU	*yeybtu*	Yersiniabactin biosynthetic protein ybtT	tga tct ccg cat tgc cgg tct tac gta aaa tct gct gcc ata aac gtg ata acg cga tct gat gta cgc t
ybtX	*yeybtx*	putative signal transducer	cgt cta tgt gac gca gag cct ggt atc ggc gct gtc tat gca gtc ctt acc cgc gct ggt gcg cgc tgc t
YE2091	*ye2091*	cytotoxic necrotizing factor	tta caa atg tgt cat ttc atc aaa agc ttc cca tgt gta ttc acc tat gcg gtg tgc caa tga ttg cgt g
YE3499	*YE3499*	type IV prepilin peptidase	cct tgg agt cag cca acg tgc aga gca tca tca cgt cca cgc aag ggc tac taa aaa gtc gta acg gtt a

continued

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Gene Name	Name of sequence spotted on microarray	Gene product	Oligonucleotide probes (5'-3')
YE3501	*YE3501*	type IV pilus integral membrane protein	cct tag gcg ttg cgc tac aga tga ttg ccg agg ttc ata ccg att ttg gca aac act ggc atc cct atg g
YE3503	*YE3503b*	putative type IV pilus protein	aga ata tgt tac tgg agg ccc aag ttc aaa ccg ctc gcc ttc agc gtc aat tgc gtg aaa acg gac tgg g
YE3503	*YE3503*	putative type IV pilus protein	ggg caa cac atc gat tga aat tat cgg cta aac cac agc ata tta ccg gtt tgg tgc gcc tta ctc gtg t
YE3506	*YE3506*	putative type IV pilus operon lipoprotein	acc cta atc gtg ttc ctg agg ttg tgc gct acg acc gct ata cct tgg tca gca gtc ggc cta ttg atg c
YE3543	*ye3543*	type III secretion protein	ata tcc gca aag agg aac cag cca atg tct cca ggc cgg taa cgc gga aca ccg ata atg ggc agg cag t
YE3543	*ye3543a*	hypothetical protein	aga atc tgc tga gcc tca cac aac ggc gac tgg agc gaa tca aac aag acc agc aga tat tgc ggc gtt c
YE3550	*ye3550*	hypothetical protein	tga taa tga gga ttg cca gtg ggc ctc taa gcg gta atg aat taa tgc tca aag cgg gta gcc atc gaa t
YE3551	*ye3551*	hypothetical protein	gcg cct gat att act tca acg ggt cac aac atg ggg atg gta tac cgt gcc ctg gca cca tta aac gga a
YE3554	*ye3554*	hypothetical protein	atg aca gcc aat ccc ggt cgg ttt agt ttc gct tgc cag cat gaa atc ttg ctg gca ccg ggt ggc tac t
YE3555	*ye3555*	hypothetical protein	tgg cca gta gtg agg ccc gct tgc aaa cac tgt tag agg aga tgt tta gtg atc cac gga tgt atg aaa t
YE3556	*ye3556*	hypothetical protein	gtg gtg aaa gtc gcc gta aca gag aaa aag gaa cct acg gat gca cca caa ctt tca gcc atc gag caa t
YE3558	*ye3558*	hypothetical protein	tga ttg cca ctt tac caa gaa ttg gta ttt cat ata cag gta cca aga aag cgc tga cag ccg agt caa c
YE3559	*ye3559*	hypothetical protein	cgc tct cac ttt ctc aga ata agg ctt ctg gag aga aag aat aca tgc ttc atg agt ata gtt tta tga c
YE3565	*YE3565*	general secretion pathway protein D	aag gca aca tca gcg tac gca gct atc aag agc tgg aac ctg acc gtt att acc cat tct tcc tga gtg t
yerA	*yeyera*	yopE regulator (yerA)	acc ggt tat cgg tgt caa agt tgg gga att cgc ctg cca tat aac aga gca tcc tgt cgg gca aat att a
yfeA	*yeyfea*	periplasmic-binding protein	gtt ttt tac ata cca tga cct tgt tta cag tcg tgg caa ttt cat tgc tga tga aca cct ctg cat ggg c
yfeC	*yeyfec*	chelated iron transport system membrane protein	ggc gac gcg cta tca atc gcg tca gaa gcg ccg tca ccc tgc ggc ctc tca ccc gga gga taa tgc atg a
yfuB	*yeyfub*	yfuB protein	atg gcc aat gta aac ccc gat gtt atc ccg atg cca ctg agt cag acc act caa cgc caa tat cag ccg c
yhcQ	*yeyhcq*	putative HlyD family secretion protein (insecticidal pathogenicity island tc-PAIye)	tca aga aga aag acg aga aat tag gta aaa aag aca gca ata caa cgc ggt att aaa cac gct gga tgc
yhp	*yeyhp1*	hypothetical protein yhp1	att aat tgc cag cgg cat aac tcg agg taa gtt gat gga ttt tca tag ccg ata taa att agt gtt att g
ylpA	*yeylpa*	lipoprotein ylpA (plasmid)	aat aaa tac atc taa aga aga aaa taa cag tta cta ctg cgc tac tgc tca ttt act gag aac gga tgt t
ymp	*yeymp1*	hypothetical protein ymp1	atg tcc att tac aac gca tac cca tta cat caa gtg atc ttg cgt cgt atc gct gtg att ctg gtg ggg t
yopB	*yeyopb*	yopB/D chaperone/regulatory protein (plasmid)	tat cgc cat gct caa cga aat ttc aag tga cac ttt aga gca act cta ctc tct tgc gtt taa cca ata c
yopB	*yeyopb2*	yop effector yopB (plasmid)	ccc ttc aga ccc aac aag tcg cgg gag aac tga agg ata aaa atg gcg ggg tga gtt ctc agg gcg tac a
yopE	*yeyope*	virulence determinant	caa gct atc act caa tta ttt caa caa ctt tcg ctg tct att cca gat act att gaa ccg gtt atc ggt g
yopH	*yeyoph*	putative yopH targeting protein	gtg gtt ttt atg cgg gca ggg atc ttg act ggt cag tct caa ctt tat gat att ttg cgg aaa aac ctc t
yopM	*yeyopm*	yop effector yopM	gta tct aat act ttt ttg caa gaa cca tta cgt cat tct tct aat tta act gag atg ccg gtt gaa gca g
yopN	*yeyopn*	control of yop release (plasmid)	tca gat cgt aaa tca gac tct ggg tca att tcg ggg aga atc tgt gca gat agt cag cgg cac tct gca g
yopO	*yeyopo*	putative targeted effector protein kinase	agc ctt tgt cag gag ctc cgg atc ttg aag gga tgc gag tgg ctg aaa ccg ata agt ttg ccg agg gcg a
yopP	*yeyopp*	Yop effector YopP	gta att cag gcg aac aat aaa tat ccg gaa atg aat ctt aat ttt gtt aca tct ccc cag gac ctt tc
yopQ	*yeyopq*	virulence plasmid protein	atg ttt att aaa gat act tat aac atg cgt gct tta tgt acc gct ctt gaa cag tcg gct cct gat aca a
yopT	*yeyopt*	yop effector yopT	atg cag aca acc ttc aca gaa ctt atg caa cag ctt ttc ctg aag ctt ggc ttg aac cat caa gtt aat g
yplA	*yeypla*	phospholipase A	ggc ctg ttc cat tag cag gct tga tga acc agg tgc aac caa tag agc agg tca aga agg aga act cta c
yplA	*yeypla*	phospholipase A	gga gaa ctc tac tcc tgt tgg gtc ttc gaa cca gct tca aaa aga atc cgc ctc cct aac tcc cac tca g
yplA	*yeypla2*	phospholipase A	atg ctg gct agc ctt gga ata tca cgt ggt gaa cgt gtt tta gct gcc agc gac aat gta ccg ata tca a
ysaC	*yeysac*	AraC family regulatory protein	tgg tcg gca agc tgt ttg atg ccg tcg ctg aac ggc tgg aga agc cga tta ttc tca gta agt tgg cgg c
ysaE	*yeysae*	similar to *Salmonella typhimurium* InvF	gca tta att att taa gtc agc gat atg gcg tct ctg cgg cct act tca ggc aat tat atc gag aga gct t
ysaH	*yeysah*	possible type III secretion system effector protein	aac aaa aac ggt agc cat gat tat ggc ctg atg cag att aat acc att aat att gat ccg cta aaa tcc c
ysaI	*yeysai*	putative virulence associated protein	ccg ctc tga tta acg ggg ccg aca cta tag atg tgt cta acc cct cgg agt tat tac aat ttc aaa gtc g
ysaJ	*yeysaj*	hypothetical protein (prokaryotic membrane lipoprotein lipid attachment site)	cgc gca ggg aaa atg gca agc tag gta att cca tta agg tgg cac gca gtg att ttg tga ccg cag tgg a
ysaK	*yeysak*	Previously sequenced as *Yersinia enterocolitica* type III secretion sytem protein YsaK	att ttg tgg tgc agc att gct gaa tgt caa atg ggg cat ctt gaa gcg ctg ggc caa aat tta ttg agc g
ysaN	*yeysan*	hypothetical protein	tta tat gac tta gaa aac atc aag gcg gaa ctg ata aca ctt gag aag aag caa cgg cag atg cag cgc c
ysaQ	*yeysaq*	Type III secretion system ysa	cgc tag cgg aaa gtc agg aaa aca cgt tag ggg atg agc aga cgc att tgc tga cta cac aac agt tgg c
ysaR	*yeysar*	Type III secretion system ysa	aac agg tac ctt ccg gcc tgg cgc tca acg gga tag cgc tgc tgt tgt cac tgt ttg tga tga tgc cgg t
ysaT	*yeysat*	putative type III secretion apparatus prote	ctt ttg gta tca agt tac tcg cgg tat ttg gct cac tgt tta tga tct ctg gtt ggc tgt ctg aca agg t
ysaU	*yeysau*	Type III secretion system ysa	atg gat tag gta gtt tgt tac aaa gaa ttt tgc ttt cac ctt ccg ata tag gtg ttg agg cat tgt tgg a

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Gene Name	Name of sequence spotted on microarray	Gene product	Oligonucleotide probes (5'-3')
ysaV	*yeysav*	type III secretion system apparatus protein	tca ttt ccc tca att tta ttg att act acg cta ttc cga tta gcg tta tcg atc agt acc agc cgt ctt a
ysaW	*yeysaw*	*Yersinia enterocolitica* type III secreted effector protein	cgg gcc gac aac aga cag caa gga aga gtg gac gcg ttt ttc tga gcg cat cct gga tgc tca agc gga t
yscA	*yeysca*	conserved hypothetical hydrophobic protein	atg agc caa att aca acg aaa cat ata aca gta tta ttt cgc cgc tgg at
yscB	*yeyscb*	hypothetical protein (plasmid)	tta ccg ttt aac tat aga taa gca tct tgt cat gct ggc tcc gca tgg ttc aga act ggt att acg cac t
yscC	*yeyscc*	type III secretion outer membrane pore, YscC/ HrcC family	tcg tct cac tga aac taa ccc act cac aga gaa tag cca tca gat atc tac cgc cga aaa agc ctt tgc c
yscD	*yeyscd*	putative type III secretion protein (plasmid)	tgt ttt tgg ttc aga ccc gtt gca gtc aga tat tgt tct ttc tga cag cga aat agc acc cgt gca ttt a
yscF	*yeyscf*	putative type III secretion protein (plasmid)	gaa gcc agc aga cgg cgc aaa caa agc ggt taa tga ctc gat agc agc gtt gaa aga gac gcc tga caa c
yscG	*yeyscg*	required for yop secretion (plasmid)	ctt tga tga acc gtg ggg act acg caa gcg cct tgc aac aag gaa ata aat cag ctt atc ctg att tgg a
yscH	*yeysch*	yop proteins translocation protein H YscH (plasmid)	aag ttc tgt ggc agc aat att atg cca gta acc ctc ctg acc atg ccg ttc ttg agg ttt tgg cga cgc c
yscI	*yeysci*	yop proteins translocation protein I YscI (plasmid)	aga aga ctt tta aga cgg cta aaa gtg act tgc aca cta agc tgg ctg ttt cag ttg ata atc cca acg a
yscK	*yeysck*	required for yop secretion (plasmid)	ggc gct gcc ttt gca gcc cca atc aca gct cga act gtt act ttg tcg cct tgg att agt tct gca tgg g
yscL	*yeyscl*	putative type III secretion protein	gcc att tgt tca aat aat acc aag taa tct ctc gct cgc ttg cgg tct gcg tat ttt gcg cgc cga aga t
yscL	*yeyscl2*	Yop proteins translocation protein L	gga ggt tta tga gca aca aaa gca gtt agg atg gca ggc tgg cat gga tga ggc gcg tac ctt aca ggc g
yscM	*yeyscm*	*Yersinia enterocolitica* yop proteins translocation protein M YscM (plasmid)	gtg agg tac tgg aac atg tga aaa ata cgg ctc tca gtc gtc acg ata ttg cct gct tat tac cac gcg t
yscN	*yeyscn*	probable type III secretion system ATP synthase (plasmid)	agg acg tgt cac tca agt gac agg aac gct att aaa agc tgt agt gcc tgg tgt gcg cat cgg tga gtt a
yscO	*yeysco*	putative type III secretion protein	atg ata cgc cgc ctg cac cgg gtt aaa gtt tta cgc gtt gaa cgt gcg gag aaa gcc atc aag act cag c
yscP	*yeyscp*	required for yop secretion (plasmid)	gca agc acg tgc cga ttt tga gca agc gct gtt gca taa taa taa ggg taa tcg tca tcc caa aga aga g
yscQ	*yeyscq*	type III secretion system protein (plasmid)	atg agt ttg tta acc ttg cca caa gcc aaa tta agt gaa ctg tcg cta cgt caa cgg ctc agc cat tat c
yscR	*yeyscr*	putative type III secretion protein (plasmid)	ggt aat ggc tac atc gtt tgt caa att tgc ggt ggt ttt ttc act act ccg caa tgc cct tgg ggt aca g
yscS	*yeyscs*	required for yop secretion (plasmid)	ggt gtt agt ggc tgc ggt ggt agg aac ttt ggt atc ttt agt aca agc ttt aac gca aat cca aga gca a
yscT	*yeysct*	putative type III secretion protein	gta aac agc tcc tcg gtg gag tat tgc tgc gta atg gta ttg tct gtt cat tgg ctc ttt atg tct atc c
yscU	*yeyscu*	putative type III secretion protein	gaa aag taa gga agt ggt ctc tac tgc gct tat cgt cgc gct gag tgc gat gtt aat ggg gct ttc tga c
yscX	*yeyscx*	Yop proteins translocation protein X	gtg agt cgc ata ata act gcc ccc cat att ggc atc gaa aaa ctg tcg gcg att agc ctg gaa gag cta t
yscY	*yeyscy*	chaperone protein yscY / Yop proteins translocation protein Y	atg aat att act tta acc aaa cga caa cag gag ttc ttg ctg ctc aac ggt tgg tta caa cta caa tgt g
ysp	*yeysp2*	similar to *Salmonella typhimurium* sipC (Cell invasion protein sipC)	aac tgt cga cgc cta aaa cga cgg ttg atc atg cgc aga tgc tga tgc agt tgc agt cgt tag agg gcc t
yspB	*yeyspb*	Type III secretion system ysa	aac atc acc gcg tta agg gtg cgc cgc gat tgg tca tgc caa aaa tga cgc tcg cac agg cca aat ccg a
yspC	*yeyspc*	membrane protein	ttt act gcc cct aag att gac cca aat ttt gca cag ttg ctg aca tcg gtt ggt gcc gag caa aat acg c
yspD	*yeyspd*	unknown	tta ttt cac tac ctc aac atg tcc cgg aac tga tgt tgg gcg cga aag ggg cac tgg cta acg ggc aca a
ysrR	*yeysrr*	response regulator protein	tcg tcg gtt gtg tca gta ata cac ccg aac tca tca cgc tac tag aaa gca acc ctg aga ttg aag tac t
ysrS	*yeysrs*	sensor kinase protein	gat ata atc tcc act gtc acc gga ata aaa ttt aag cca gta ttc agt aac aac gac aga gaa tcc gtt a
yst	*yeyst*	enterotoxin	acc agc cga agt cag tag tga ttg gga ttg ctg cga tgt atg ttg caa tcc tgc ctg tgc ggg ttg cta g
yts	*yeyts*	yts1K protein	tta gat aag gac gac ata tcc cgt atc acg gct gga agc gag gct acg ctg tat cag aca agc cgc ccg g
yts1C	*yeyst1c*	general secretion pathway protein C	act acg aca aga aat att gtt gtg gcg tta ctg ctg gtc att ttg att tta ctg cgt att gag cat cat c
yts1E	*yeyst1e*	general secretion pathway proteinE (2)	atg aac cag gca gtc aca cta cag cca cag ttg cct ttt gcc tgg gta caa caa tat ggg gtg ctg tat c
yts1F	*yeyst1f*	general secretion pathway protein F	gct tgg gca aca act gac acc tat cag cct gac gcc tga aaa tga tgc gcc acg tgg gtt ttc act ctc c
yts1G	*yeyst1g*	general secretion pathway protein G precursor	tta cct tac tgg aaa tca tgg tgg tca ttg tca ttc ttg gcg tgc ttg cca gcc tca cca ttc cca gcc t
yts1H	*yeyst1h*	general secretion pathway protein H	tta cct taa tgg aaa tta tgc tgg tac tag tgt tat tgg gct gca tgg cca agc tgg cgc tgg gca ctt t
yts1I	*yeyst1i*	general secretion pathway protein I precursor	tac cgg tga aca agt cag gaa tct aag tca tct tga aaa gag aca att tgc cgc ttg ggt cgc tga aaa c
yts1J	*yeyst1j*	putative general secretion pathway protein J precursor	taa cag tga att gac acg tca aca cgc cac cag gct gac tga tat tca acg tac ttt tac ctt gat gga g
yts1K	*yeyst1k*	putative general secretion pathway protein K	cat gat gac cat tac agc cgt caa tat gaa tga cca ctg gct acg agc gtt taa tcg ggc tac cag cac c
yts1L	*yeyst1l*	putative general secretion pathway protein L	gaa aaa taa aac atc ttc cga cga att att tat atc tct tgc tga aag tgc ggg aca gcc agt tca ttg g
yts1M	*yeyst1m*	putative general secretion pathway protein M	tat cag cat caa agc aca caa aac cta cac agc gcc cag cgg gat ttc gcc gcg tta ctg caa caa caa g
yts1O	*yeyst1o*	type 4 prepilin-like proteins leader peptide processing enzyme	ttg tat gct atc caa aca atc gct ccg ctt tgg tgg agc agt ctg gcc tta ctg ggt ctg tgt gtc ggc a
yts1S	*yeyst1s*	putative secretion protein	gca gca gat caa aca att gtc cgc att agt tgc tgg ggc gca tta tct aca gaa aga ttg cca gat aaa g