



**UNIVERSITI PUTRA MALAYSIA**

**ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF  
YERSINIA SPP FROM MEAT AND MEAT PRODUCTS**

**AHMAD ZAINURI MOHD. DZOMIR.**

**FSTM 2005 3**

**ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF *YERSINIA*  
SPP FROM MEAT AND MEAT PRODUCTS**

**By**

**AHMAD ZAINURI MOHD. DZOMIR**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia  
In Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

**May 2005**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirements for the degree of Doctor of Philosophy

## **ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF *YERSINIA* SPP FROM MEAT AND MEAT PRODUCTS**

By

**AHMAD ZAINURI MOHD. DZOMIR**

**May 2005**

**Chairman: Professor Gulam Rusul Rahmat Ali, PhD**

**Faculty: Food Science and Technology**

Three hundred and twenty two samples comprising of beef (94), chicken parts (114), pork (11), beef burger (47), chicken burger (30), chicken nugget (1), chicken frankfurter (10), chicken carcass (20) and pork frankfurter (5) were examined for the presence of *Yersinia*. Samples were enriched in phosphate-buffered-saline at 25°C for 48h. Enriched samples were treated with 0.5% potassium hydroxide (KOH) solution and then streaked onto Cefsulodin-Irgasan-Novobiocin (CIN) agar plates. 1/94 (1.1%), 16/47 (34.0%), 6/14 (5.3%), 1/30 (3.3%) and 1/20 (5.0%) of beef, beef burgers, chicken parts, chicken burgers and chicken carcass samples were contaminated with *Yersinia* spp. respectively. *Yersinia* spp. were not isolated from pork, chicken nugget and chicken frankfurter samples. Fifty-three

isolates of *Yersinia* spp. were isolated from 25 (7.7%) positive samples and identified as *Y. enterocolitica* (29), *Y. frederiksenii* (18), *Y. kristensenii* (3) and *Y. intermedia* (3). Highest numbers of positive samples were obtained from Selangor (86.2%), followed by Negeri Sembilan (6.9%). 3.4% of the positive samples were obtained from Kuala Lumpur and overseas. In this study, *Y. enterocolitica* was defined as non sensu stricto on the basis of biochemical properties not strictly fitting according to the scheme used for classification at the genus level. They were biochemically atypical, including Simmon's citrate-positive and Voges-Proskauer-negative. All *Y. enterocolitica* isolates were grouped into biotype 1A based on reaction to D-xylose, nitrate reduction and pyrazinamidase. The four related species: *Y. frederiksenii*, *Y. intermedia* and *Y. kristensenii* were readily distinguishable by sucrose, melibiose, rhamnose and raffinose fermentation. The result of serotyping of twenty *Y. enterocolitica* showed that eleven of them belonged to serotype O:52,53; one isolate belong to serotype O:41,42 and nine were untypable, delineating the isolates from pathogenic serotypes. In addition, Polymerase Chain Reaction (PCR) analysis indicated that *Y. enterocolitica* examined did not possessed any of the virulence marker genes that are characteristics of pathogenic strains. Antibiotic susceptibility analysis showed there was no difference in the susceptibilities of the four *Yersinia* species towards ampicillin, penicilin, cephalotin, bacitracin and chloramphenicol. All isolates (100%) were resistant to ampicilin, penicilin and cephalotin but all (100%) were sensitive to chloramphenicol. 1.96, 3.92, 5.88, 9.80 and 29.41%

of *Yersinia* isolates were resistant to gentamicin, nalidixic acid, streptomycin, tetracycline and cefaporozone, respectively. 98.04% of the isolates which were resistant to carbenicillin demonstrated weak activity of carbenicillin against the *Yersinia*. Multiple Antibiotic Resistance (MAR) index ranged from 0.36 to 0.64. Three plasmid patterns were observed among the *Yersinia* isolated. 31 (60.78%) of the isolates carried single plasmid and 20 (39.22%) of the isolates did not carry any plasmid. 21 (41.18%), 8 (15.69%) and 2 (3.92%) of the isolates were harbor 54, 32 and 2.7 MDal plasmid size, respectively. The dendrogram obtained by comparative analysis of the pulsed field electrophoresis patterns clustered biotype 1A into three clusters (A, B and C). The PFGE result indicated that they (biotype 1A) were different from the pathogenic strains (control strains).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PEMENCILAN, PENGENALPASTIAN DAN PENCIRIAN *YERSINIA* SPP.  
DARIPADA DAGING DAN PRODUK DAGING**

Oleh

**AHMAD ZAINURI MOHD. DZOMIR**

**Mei 2005**

**Pengerusi: Profesor Gulam Rusul Rahmat Ali, PhD**

**Fakulti: Sains dan Teknologi Makanan**

Tiga ratus tiga puluh dua sampel daging lembu (94), daging ayam (114), daging khinzir (11), burger lembu (47), burger ayam (30), nugget ayam (1), frankfurter ayam (10), bilasan daging ayam (20) and frankfurter khinzir (5) telah diperiksa bagi mengesan kehadiran *Yersinia*. Agar cefsulodin-irgasan-novobiocin (CIN) telah diinokulasikan dengan sampel yang telah dinkubasi dalam phosphate-buffered-saline (PBS) 1/15M, pH 7.6 pada suhu 25°C selama 48 jam setelah ia dirawat dengan 0.5% larutan potassium hydroxide (KOH). Frekuensi sampel yang positif adalah seperti berikut; daging lembu (1.1%), burger lembu (34.0%), daging ayam (5.3%), burger ayam (3.3%) dan bilasan daging ayam (5.0%). Daging khinzir, nugget ayam dan frankfurter ayam didapati tidak mengandungi *Yersinia*. Lima puluh tiga kultur *Yersinia*

telah dipencil dari 25 (7.7%) sampel dan dikenalpasti sebagai *Y. enterocolitica* (29), *Y. frederiksenii* (18), *Y. kristensenii* (3) and *Y. intermedia* (3). Taburan *Yersinia* yang dipencil menurut tempat asal sampel mendapati Selangor (86.2%) sebagai penyumbang utama kepada kontaminasi. Ini diikuti dengan Negeri Sembilan (6.9%) dan 3.4% bagi setiap sampel dari Wilayah Persekutuan dan sampel yang diimport. *Y. enterocolitica* dalam kajian ini dikatakan *non sensu stricto* berdasarkan ciri-ciri biokimia yang kurang menepati corak klasifikasi bagi tahap genus. Ia menunjukkan ciri-ciri biokimia yang atipikal, termasuk positif bagi ujian sitrat Simmon dan negatif bagi ujian voges-proskauer. Kesemua pencilan *Y. enterocolitica* digolongkan di dalam biotip 1A menurut keputusan ujian-ujian D-xylose, penurunan nitrat dan pyrazinamidase. Keempat-empat spesis *Yersinia*: *Y. frederiksenii*, *Y. intermedia* dan *Y. kristensenii* boleh dibezakan dengan mudah berdasarkan keputusan ujian fermentasi sukrosa, melibiosa, rhamnosa dan raffinosa. Percubaan untuk mengaitkan kadar pemencilan dengan tempoh pemencilan bulanan didapati tidak berjaya. Keputusan ujian serotip bagi dua puluh kultur *Y. enterocolitica* menunjukkan sebelas darinya tergolong dalam serotip O:52,53; satu kultur O:41,42 dan sembilan kultur tidak boleh diserotipkan. Ini menunjukkan mereka tidak tergolong dalam serotip yang virulen. Tambahan pula, analisis PCR menunjukkan kesemua *Y. enterocolitica* yang diuji tidak mempunyai gen virulen seperti yang dipunyai oleh kultur yang patogen. Ujian kerintangan kepada antibiotik menunjukkan tiada perbezaan kerintangan yang ketara ditunjukkan oleh keempat-empat spesis *Yersinia* terhadap

ampicilin, penicilin, cephalotin, bacitracin dan chloramphenicol dimana kesemuanya (100%) rintang terhadap ampicilin, penicilin dan cephalotin. Manakala kesemuanya (100%) adalah sensitif terhadap chloramphenicol. Gentamicin, nalidixic acid, streptomycin, tetracycline dan cefaporozone menunjukkan aktiviti berkesan dimana hanya 1.96, 3.92, 5.88, 9.80 dan 29.41% *Yersinia* yang rintang. Sebanyak 98.04% kerintangan menandakan aktiviti tidak berkesan carbenicilin terhadap *Yersinia*. Analisis indeks MAR yang dihitung menunjukkan julat antara 0.36 hingga 0.64. Tiga corak plasmid diperhatikan: 31 (60.78%) membawa satu plasmid dan 20 (39.22%) tidak mempunyai plasmid. 21 (41.18%), 8 (15.69%) dan 2 (3.92%) masing-masing membawa plasmid bersaiz 54, 32 dan 2.7 MDal. Dendrogram yang diperolehi dari analisis perbandingan corak elektroforesis pulsed-field menggolongkan biotip 1A kepada tiga kumpulan besar (A, B dan C). Keputusan ini menunjukkan bahawa biotip 1A adalah berbeza dari kultur patogenik (kultur kawalan) setelah method PFGE digunakan.



## **ACKNOWLEDGEMENTS**

All praise to Almighty Allah, the Merciful and the Benevolent. Had it not been due to His will and favour, the completion of this study would not have been possible.

I would like to acknowledge the following people for making this study possible.

First I would like to express my sincere appreciation to Prof. Gulam Rusul Rahmat Ali, Prof. Son Radu and Dr. Sahilah Abd Mutalib for their invaluable guidance, advice, supervision and patience in supervising this study.

I would also like to extend my sincere appreciation to Dr. Maznah Ahmad and Dr. Akma Ngah Hamid, Regional Veterinary Diagnostic Laboratory and Dr. Rafal Gierzynski, National Institute of Hygiene, Poland. Thank you as no words can express my gratitude for the help and support.

I also wish to thank Dr. Astrid Waage and Dr. A. Rakin the cultures contributor. I realize that the study could not be completed without the help of Dr. Elisabeth Carniel, Institut Pasteur of Paris who helped me with the serotyping and for that I will always be grateful to Dr. Carniel.

My sincere thanks also goes to members of the laboratory and finally to my family for their belief in me. Their never-ending support gave me the strength to complete the study.

May Allah Bless All of You

**AHMAD ZAINURI MOHD. DZOMIR**

## TABLE OF CONTENTS

	Page
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL SHEETS	xi
DECLARATION FORM	xiii
LIST OF TABLES	xvii
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxi
 CHAPTER	
 1 <b>INTRODUCTION</b>	 1
Objectives of the present study	5
 2 <b>LITERATURE REVIEW</b>	 6
The Genus <i>Yersinia</i>	6
Brief historical background	6
Common characteristics	6
<i>Yersinia enterocolitica</i> sensu stricto and related species	8
Variability within species	12
Biotype heterogeneity	12
Serology	13
Epidemiology and geographical distribution	15
Growth and survival in foods	16
Occurrence in retail meat and meat products	18
Virulence characteristics	19
Relationship between bio-sero and pathogenicity	19
Clinical manifestations	21
Mechanism	22
Test for pathogenicity	25
Clinical significance of biotype 1A	28
Foodborne outbreaks	30
Recovery and detection methodology	33
Conventional	33
Molecular	38
Typing for epidemiological study of <i>Yersinia enterocolitica</i>	40
Restriction endonuclease of virulence plasmid (REAP)	41
Randomly amplified polymorphic DNA (RAPD)	42
Multi locus enzyme electrophoresis (MLEE)	44



	Pulsed field gel electrophoresis (PFGE)	44
3	<b>INCIDENCE OF <i>YERSINIA</i> SPP IN MEATS AND MEAT PRODUCTS</b>	47
	Introduction	47
	Materials and Methods	49
	Collection of samples	49
	Enrichment methods	51
	Isolation and identification	52
	Bacterial strains for characterization of <i>Y. enterocolitica</i> by biotyping, serotyping and polymerase chain reaction (PCR)	53
	Pyrazinamidase test	54
	Salicin-esculin hydrolysis test	54
	D-xylose fermentation	55
	Lipolytic activity	55
	Indole test	56
	Nitrate reduction test	56
	Serotyping	57
	Detection of the <i>virF</i> , <i>yadA</i> , <i>myfA</i> , <i>ail</i> , and <i>ystA-C</i> gene by PCR amplification in <i>Y. enterocolitica</i> to detect pathogenicity	57
	Results	59
	Identification	59
	Prevalence of <i>Yersinia</i> in food samples	60
	Distribution of <i>Yersinia</i> according to origin of samples	62
	Biotyping	63
	Serotyping	64
	Polymerase chain reaction (PCR)	64
	Discussion	66
4	<b>ANTIBIOTIC SUSCEPTIBILITY AND PLASMID PROFILING OF <i>YERSINIA</i> SPP</b>	74
	Introduction	74
	Materials and Methods	76
	Antibiotic susceptibility testing (disk diffusion test)	76
	Multiple antibiotic resistance (MAR) indexing of <i>Yersinia</i> isolates	78
	Plasmid analysis	78
	Electrophoresis	79
	Results	80
	Susceptibility of <i>Yersinia</i> isolates	80
	Multiple resistance to antibiotics	80
	Plasmid profiles of <i>Yersinia</i> food isolates	82

	Comparison of antibiotic susceptibility of <i>Yersinia</i> with and without plasmid	88
	Discussion	89
5	<b>MOLECULAR CHARACTERIZATION OF <i>YERSINIA</i> SPP. BY PULSED FIELD GEL ELECTROPHORESIS (PFGE)</b>	95
	Introduction	95
	Materials and Methods	96
	Bacterial strains	96
	Preparation of genomic DNA	97
	Genomic DNA digestion by restriction enzyme	97
	Pulsed field gel electrophoresis	98
	Comparative analysis of electrophoresis patterns	98
	Results	99
	PFGE analysis of <i>Y. enterocolitica</i> after genomic digestion with <i>Xba</i> I and <i>Not</i> I	99
	Comparison between the use of <i>Xba</i> I and <i>Not</i> I to discriminate food from control isolates of <i>Y. enterocolitica</i>	103
	Homogeneity of the pulsotypes among pathogenic <i>Y. enterocolitica</i> , especially serotype O:3/4 (control strains)	104
	High degree heterogeneity of pulsotypes in <i>Y. enterocolitica</i> biotype 1A	104
	The pulsotypes of biotype 1A strains are closely related to their serotypes	105
	PFGE analysis of <i>Y. frederiksenii</i> , <i>Y. intermedia</i> and <i>Y. kristensenii</i> after genomic digestion with <i>Xba</i> I	105
	Discussion	109
6	<b>GENERAL DISCUSSION</b>	114
7	<b>CONCLUSION</b>	117
	<b>REFERENCES</b>	118
	<b>APPENDICES</b>	139
	<b>BIODATA OF THE AUTHOR</b>	145

## LIST OF TABLES

Table		Page
2.1	Biochemical differentiation of major groups of Enterobacteriaceae	7
2.2	Biochemical characteristics of <i>Yersinia enterocolitica</i> sensu stricto (Bercovier <i>et al.</i> , 1980a)	9
2.3	Characteristics of <i>Y. enterocolitica</i> and related species (Bottone, 1997)	11
2.4	<i>Y. enterocolitica</i> biogrouping scheme (Wauters <i>et al.</i> , 1987)	13
2.5	Virulence of <i>Y. enterocolitica</i> correlated with biogroup, serogroup and ecologic and geographic distribution (Bottone <i>et al.</i> , 1997)	15
2.6	Correlation between serogroup, biotype and pathogenicity of <i>Y. enterocolitica</i> (Bercovier <i>et al.</i> , 1980a)	20
2.7	Nucleotide and amino acid sequence comparison between structural genes and precursor proteins among <i>Yersinia</i> -STs (Ramamurthy <i>et al.</i> , 1997)	28
2.8	Summary of procedures used to recover <i>Yersinia</i> from food	35
2.9	Detection of <i>Yersinia enterocolitica</i> by PCR	39
3.1	Distribution of samples according to their origin	51
3.2	Sequence of primers used in the polymerase chain reaction study	59
3.3	Number of samples positive for <i>Yersinia</i>	61
3.4	Isolation of <i>Yersinia</i> from meat and meat products samples over 8 months period of study	62



3.5	Distribution of <i>Yersinia</i> isolates according to origin of samples	63
3.6	<i>Yersinia enterocolitica</i> serovars and biotypes isolated from different types of foods	65
4.1	Groups of antimicrobial agents used in this study and their respective mechanism of action	77
4.2	The distribution of antimicrobial resistance of <i>Yersinia</i> isolates	81
4.3	Susceptibility testing of resistant strains to multiple antimicrobial agents among <i>Yersinia</i> isolates	81
4.4	Multiple antibiotic resistance index of <i>Yersinia</i>	82
4.5	The antibiotic resistance, plasmid profiling for all <i>Yersinia</i> isolates	85
4.6	Plasmid pattern of <i>Yersinia</i>	87
4.7	Antibiotic susceptibility of <i>Yersinia</i> with and without plasmid DNA	88

## LIST OF FIGURES

Figure		Page
2.1	Production of heat stable enterotoxin (Y-ST) <i>in vitro</i> by <i>Y. enterocolitica</i> and related species at 4, 22 and 37°C as determined by the infant mouse assay. YE O:3, <i>Y. enterocolitica</i> segroup O:3; YE, non-pathogenic <i>Y. enterocolitica</i> ; YK, <i>Y. kristensenii</i> ; YF, <i>Y. frederiksenii</i> ; YI, <i>Y. intermedia</i> ; YA, <i>Y. aldovae</i> (Kapperud, 1982)	23
4.1	Plasmid profiles of <i>Y. enterocolitica</i> isolates. Lane 1, MYE1; 2, MYE2; 3, MYE3; 4, MYE4; 5, MYE5; 6, MYE6; 7, MYE7; 8, MYE8; 9, MYE9; 10, MYE10; 11, MYE11; 12, MYE12; 13, MYE13; 14, MYE14 and 15, molecular weight size markers, <i>E. coli</i> strain V517 harbors eight plasmids with molecular sizes range from 1.4 to 35.8 MDa (Macrina <i>et al.</i> , 1978)	83
4.2	Plasmid profiles of <i>Y. enterocolitica</i> isolates. Lane 2, MYE15; 3, MYE16; 4, MYE17; 5, MYE18; 6, MYE19; 7, MYE20; 8, MYE21; 9, MYE22; 10, MYE23; 11, MYE24; 12, MYE25; 13, MYE26; 14, MYE27; and 15, molecular weight size markers, <i>E. coli</i> strain V517 harbors eight plasmids with molecular sizes range from 1.4 to 35.8 Mda (Macrina <i>et al.</i> , 1978)	84
4.3	Plasmid profiles of <i>Y. frederiksenii</i> isolates. Lane 1, MYF28; 2, MYF29; 3, MYF30; 4, MYF31; 5, MYF32; 6, MYF33; 7, MYF34; 8, MYF35; 9, MYF36; 10, MYF37; 11, MYF38; 12, MYF39; 13, MYF40; 14, MYF41 and 15, molecular weight size markers, <i>E. coli</i> strain V517 harbors eight plasmids with molecular sizes range from 1.4 to 35.8 Mda (Macrina <i>et al.</i> , 1978)	84
4.4	Plasmid profiles of <i>Y. intermedia</i> isolates. Lane 1, MYI46; 2, MYI47; 3, MYI48; <i>Y. kristensenii</i> , 4, MYK49; 5, MYK50; 6, MYK51; <i>Y. frederiksenii</i> , 7, MYF43; 8, MYF44; 9, MYF45; 10, molecular weight size markers, <i>E. coli</i> strain V517 harbors	



	eight plasmids with molecular sizes range from 1.4 to 35.8 MDa (Macrina <i>et al.</i> , 1978)	85
5.1	Dendrogram obtained by <i>Xba</i> I demonstrating the genetic relationship of the pulsotypes observed from 39 test and control isolates of <i>Y. enterocolitica</i>	101
5.2	Dendrogram obtained by <i>Not</i> I demonstrating the genetic relationship of the pulsotypes observed from 39 test and control isolates of <i>Y. enterocolitica</i>	102
5.3	Dendrogram obtained by <i>Xba</i> I demonstrating the genetic relationship of the pulsotypes observed from 18 test isolates of <i>Y. frederiksenii</i>	106
5.4	Dendrogram obtained by <i>Xba</i> I demonstrating the genetic relationship of the pulsotypes observed from 3 test isolates of <i>Y. intermedia</i>	107
5.5	Dendrogram obtained by <i>Xba</i> I demonstrating the genetic relationship of the pulsotypes observed from 3 test isolates of <i>Y. kristensenii</i>	108

## LIST OF ABBREVIATIONS

DNA	deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphates
EDTA	ethylenediamine tetra-acetic acid
EtBr	ethidium bromide
G	Gram
H	hour(s)
HCl	hydrochloric acid
KAc	potassium acetate
KCl	potassium chloride
M	Molarity
MAR	multiple antibiotic resistance
MDa	Megadalton
MgCl <sub>2</sub>	magnesium chloride
Mg	Milligram
Min	Minutes
ml	Milliliter
mm	Millimeter
mM	Millimolar
μg	Microgram
μm	Micrometer
μl	Microliter



Mol	Mole
N	Normal
NaCl	sodium chloride
NaOH	sodium hydroxide
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
R	Resistant
RAPD	random amplification of polymorphic DNA
RNA	ribonucleic acid
Rpm	revolution per minute
rRNA	ribosomal ribonucleic acid
S	Susceptible
subsp.	Subspecies
SDS	sodium dodecyl sulphate
spp.	Species
TBE	tris-borate EDTA electrophoresis buffer
Tris	tris (hydroxymethyl) methylamine
Uv	ultra violet
V	Volts
WHO	World Health Organization
w/v	weight per volume
v/v	volume per volume
>	more than

%	Percentage
°C	degree celcius
P+	with plasmid
P-	without plasmid

## CHAPTER 1

### INTRODUCTION

*Yersinia enterocolitica* and *Yersinia enterocolitica*-like bacteria, including *Y. frederiksenii*, *Y. kristensenii*, *Y. intermedia*, *Y. aldovae*, *Y. rohdei*, *Y. mollaretti* and *Y. bercovieri* constitute a fairly heterogeneous group of bacteria which includes both well-established pathogens and range of environmental or non-pathogenic strains. *Yersinia* is a gram negative, non-spore forming, facultative anaerobic bacterium that can multiply at refrigeration temperatures, 0-4°C (Bottone, 1997; Bottone, 1999).

There has been a steady increase in the number of isolations of *Y. enterocolitica* in recent years, not only from clinical materials but also from water and foods. *Y. enterocolitica* and related species are common in many types of foods such as milk (Moustafa *et al.*, 1983; Franzin *et al.*, 1984; Toora *et al.*, 1989; Ibrahim and Rae, 1991), meat and meat products (Logue *et al.*, 1996; Fukushima *et al.*, 1997; Johannessen *et al.*, 2000; Ramirez *et al.*, 2000), also in other animal associated products (Velazquez *et al.*, 1993). *Yersinia* is of particular importance for the safety of consumers, because it is capable of growing in raw meat and meat products and remains viable for long periods at refrigeration temperatures (Hudson and Mott, 1993; Johnson *et al.*, 1982; Myers *et al.*, 1982). As the number of *Yersina* in foods is usually

low and there is often great variety of background flora, direct isolation on selective plating media is seldom successful. Isolation methods usually involve enrichment of the sample followed by plating onto selective agar media and confirmation of typical colonies (Hoorfar and Holmvig, 1999; Johannessen *et al.*, 2000). In addition, alkalotolerance in *Y. enterocolitica* compared to other bacteria has been used to reduce the level of background flora and made detection of *Y. enterocolitica* easier (Jiang *et al.*, 2000).

Unlike *Salmonella* and *Shigella* species, which are intrinsic pathogens and essentially all strains can cause enteric infections, there is strain-to-strain variation in the pathogenicity of *Y. enterocolitica*. A number of studies have shown an excellent correlation between the serotype and biotype of *Y. enterocolitica*. Biotype 1A usually comprises avirulent strains and encompasses a wide range of serotypes (O:5; O:6,30; O:6,31; O:7,8; O:10) as well as non-typable O strains (Tennant *et al.*, 2003). Whereas biotype 1B and biotype 2-5 include strains that are potentially pathogenic for man and animal. These biotype belonged to only a few serotypes such as O:3; O:5,27; O:8; O:9 of *Y. enterocolitica sensu stricto*. An accurate designation of pathogenic *Y. enterocolitica* strains need to take into account both the biotype and the serotype of a strain. The two traits are linked closely. Biotype 4 is associated with serotype O:3 (4/O:3), biotype 2 with serotype O:9 and, less frequently O:5, and biotype 3 with serotype O:3 and O:5. Therefore, the