International Journal of Medical Microbiology 301 (2011) 556–561

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.de/ijmm

The pathogenic potential of *Yersinia enterocolitica* 1A

Julia Batzilla, Juergen Heesemann, Alexander Rakin*

Max von Pettenkofer-Institute, LMU, Pettenkoferstr. 9a, 80336 Munich, Germany

ARTICLE INFO

Article history:
Received 15 March 2011
Received in revised form 4 May 2011
Accepted 9 May 2011

Keywords:
Yersinia
Genome comparison
Pathogenicity
BT 1A
MyfA

ABSTRACT

Yersinia enterocolitica 1A strains are generally considered apathogenic. However, besides environmental sources, foods and animals, they are repeatedly isolated from patients with gastrointestinal symptoms typical to those evoked by *Yersinia* of the virulent 1B and 2–4 biotypes. Also, at least 2 gastrointestinal outbreaks associated with 1A strains have been reported. There is a general controversy concerning the pathogenic potential of 1A isolates of clinical and non-clinical origin. To address the 1A puzzle, we have determined the genome sequences of 2 1A strains, a nosocomial O:5 and environmental O:36 isolates, and compared them to each other and to O:8/1B and O:3/4 representatives of the virulent serobiotypes.

1A isolates have mosaic genomes and share genes both with serobiotypes O:8/1B and O:3/4 that implies their common descent. Besides the pYV virulence plasmid, 1A strains lack the classical virulence markers, like the Ail adhesin, the YstA enterotoxin, and the virulence-associated protein C. However, they still possess genes encoding such known and suspect virulence-associated determinants like the YstB enterotoxin, the InvA invasin, the mucoid *Yersinia* factor MyfA, and the enterochelin utilisation *fepBDGC*|*fepA*|*fes* gene cluster. In contrast to previous studies, we have found that the strains of the 1A group possess the MyfA antigen although with limited similarity to the highly conserved MyfA in the virulent serobiotypes. In turn, the MyfB chaperone coevolved with the MyfA fibrillae, while the MyfC usher retains 90% identity to its MyfC counterparts in O:3/O:8 group. The only notable difference between clinical and non-clinical 1A strains was the presence of a truncated Rtx toxin-like gene cluster and remnants of a P2-like prophage in the hospital O:5 isolate.

Taken together, *Y. enterocolitica* BT 1A group represents opportunistic pathogens whose opportunity to establish infection seems to rely mainly on the state of the host defence system. However, presence of known and putative virulence-associated features shared with the pathogenic serobiotypes compels to reconsider properly the pathogenic potential of this group of emerging pathogens.

Introduction

The zoonotic pathogen *Yersinia enterocolitica* is associated with 1–3% cases of acute enteritis followed by erythema nodosum and reactive arthritis (Bottone, 1999). *Y. enterocolitica* biotypes (BT) 1B, 2, 3, and 4 are known human pathogens, while BT 1A strains are believed to be apathogenic. BT 1A is the most heterogeneous yersiniae biotype and encompasses a wide range of serotypes with O:5, O:6,30, O:6,31, O:7,8 being the most often isolated ones.

BT 1A strains are considered non-pathogenic because they lack such established classical virulence-associated determinants of the pathogenic biotypes like the virulence-associated pYV plasmid, Ail, the Ysa type 3 secretion system, and the high-pathogenicity island encoding yersiniabactin iron uptake system in biotype 1B (Revell and Miller, 2001). The absence of the latter renders BT 1A avirulent for mice. However, despite the lack of the identifiable virulence determinants in BT 1A strains, these bacteria are repeatedly isolated not only from healthy individuals, but also from patients with gastrointestinal complaints and evoke human disease with symptoms indistinguishable from that of the pathogenic biotypes (Burnens et al., 1996; Scheftel, 2002). Besides asymptomatic and symptomatic humans, BT 1A strains are frequently isolated from various mammals, birds, fish as well as other environmental sources and foods (Tennant et al., 2003a).

Although most reports of BT 1A-associated disease cases are sporadic, at least 2 outbreaks of gastrointestinal infection due to BT 1A yersiniae have been reported. The first nosocomial outbreak due to *Y. enterocolitica* serotype O:5 BT 1A (O:5/1A), which involved 9 patients in Canada was reported by Ratnam et al. (1982). Greenwood and Hooper (1990) isolated *Y. enterocolitica* 1A, serotype O:10, from 19 paediatric patients in a hospital in England. Moreover, it becomes clear that the BT 1A strains are becoming the dominating pathogenic yersiniae (58% of the reported cases) in Commonwealth of Nations countries from the end of the 20th century (McNally et al., 2004), thus surpassing O:3/4 strains. Also, a recent case–control study demonstrated that the BT 1A strains

* Corresponding author. Tel.: +49 089 51605275; fax: +49 089 51605223.
E-mail address: rakin@mvp.uni-muenchen.de (A. Rakin).

1438-4221/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijmm.2011.05.002

Table 1
Presence of the classical virulence-associated genes in strains of this study.

Gene	Highly virulent O:8/1B	Virulent O:3/4	Clinical isolate O:5/1A	Non-clinical isolate O:36/1A
ystA	+	+	–	–
ystB	–	–	+	+
invA (YE2564, Y11_14481)	+	+	+	+
myfA (YE1452, Y11_03301)	+	+	+, less conserved	+, less conserved
ymoA (YE3115, Y11_20331)	+	+	+	+
hreP (YE2568, Y11_14541)	+	+	+	+
fepBDGCfesfepA cluster (YE3618–3624)	+	–	+	+
ail (YE1820, Y11_00221)	+	+	–	–
Virulence-associated *vapC* and *vagC* (YE4141/2, Y11_30421/11)	+	+	–	–

constitute the majority of *Y. enterocolitica* isolated from diarrhoeic patients in Finland (Huovinen et al., 2010). Hence, BT 1A strains represent a rapidly emerging group of human pathogens.

There are several controversial reports on the pathogenicity of clinical and non-clinical BT 1A strains (Grant et al., 1998; Tennant et al., 2003b, 2005; Thoerner et al., 2003). On one hand, it has been shown that BT 1A strains of clinical origin differ significantly from non-clinical isolates in their ability to penetrate epithelial cells, survive within macrophages, and colonise the intestinal tract of mice (Tennant et al., 2003a). On the other hand, there is evidence that infections with BT 1A yersiniae of low pathogenic potential are more common in individuals that are generally predisposed to infections (Ratnam et al., 1982; Bhagat and Virdi, 2011). So most probably the pathogenicity of the BT 1A group might be mainly an attribute of the host that fails to resist the attack of a relatively innocuous agent.

Due to the growing clinical and epidemiological significance of *Y. enterocolitica* 1A strains, we initiated a whole-genome comparison of two BT 1A isolates, a clinical nosocomial strain [strain NF-O, serogroup O:5 (Ratnam et al., 1982)] and a non-clinical environmental one isolated from water in Japan [strain IP2222, serogroup O:36 (Grant et al., 1999)]. Both in turn were matched up to the representatives of known pathogenic serobiotypes (highly pathogenic O:8/1B and low-pathogenic O:3/4).

Materials and methods

Strains and DNA preparation

The *Y. enterocolitica* subsp. *palearctica* [Neubauer serobiotype O:5/1A strain NF-O, a clinical nosocomial outbreak isolate from Newfoundland (Ratnam et al., 1982)], and a non-clinical environmental O:36/1A strain IP2222 isolated from water in Japan [obtained from Wauters, Institute Pasteur (Grant et al., 1999; Tennant et al., 2003b)] were used for high coverage draft genome sequencing (see below). DNA was prepared using NucleoBond® AXG of Macherey-Nagel (Düren, Germany) following the manufacturer's instructions.

Genome sequencing and bioinformatics

The draft high coverage genome sequences of *Y. enterocolitica* serobiotype O:5/1A strain NF-O and O:36/1A strain IP2222 were obtained in cooperation with BGI-Hongkong Co., Hong Kong. We used high-throughput Illumina sequencing technology to conduct paired-end sequencing for DNA samples and constructed a 500-bp library with extended data of 650 (NF-O)-1000 (IP2222) Mb, and a 2-kbp library of about 900 Mb each. Genome assembly results in 10 large scaffolds and 74 contigs for IP2222 and 14 scaffolds and 97 contigs for NF-O. Genome coverage based on reads mapping was 99% for both genomes. For protein comparisons, the genome sequences were annotated using the RAST server (Aziz et al., 2008). Genome comparisons have been done using SEED (Overbeek et al., 2005), Mauve (Darling et al., 2010), Multalign (http://multalin.toulouse.inra.fr/multalin/), and other standard comparison tools. SEED was also used to determine orthologous proteins between *Y. enterocolitica* subsp. *palearctica* Y11 (O:3/4) and *Y. enterocolitica* subsp. *enterocolitica* 8081 (O:8/1B), using the standard parameters. The locus tags YE (O:8) and Y11_ (O:3) followed by numbers refer to the annotated genes that can be found under the accession numbers NC_008800 and FR729477, respectively.

Both draft genomes are deposited as whole-genome sequence projects in the EMBL sequence database as contigs under CACY01000001–CACY01000097 (97 contigs, NF-O) and CACZ01000001–CACZ01000074 (74 contigs, IP2222).

Results

General characteristics of Y. enterocolitica biotype 1A

To address the enigmatic behaviour of the emerging *Y. enterocolitica* BT 1A strains, we have determined genome sequences of 2 isolates, namely, a hospital O:5/1A isolate (NF-O) that caused a nosocomial outbreak of diarrhoeal disease in Newfoundland, Canada, and an environmental non-pathogenic strain of O:36/1A (IP2222). These sequences were compared to each other and to the genomes of the low-pathogenic *Y. enterocolitica* O:3/4 and a highly virulent, mouse-lethal O:8/1B strain.

For strain NF-O, we maintained 14 large scaffolds with 85.55% coding regions and an overall GC content of 47.08%. The draft sequence genome size was 4,695,527 bp. In the case of the environmental strain IP2222, genome sequencing yielded 10 large scaffolds with 86.16% coding sequences, a GC content of 47.14%, and a draft genome size of 4,796,259 bp. Both genomes were annotated with RAST (Aziz et al., 2008), reporting 4439 putative proteins for O:5 and 4478 protein coding sequences for O:36. From those annotated proteins, 756 and 865 proteins are designated hypothetical (known also as ORFans), respectively.

To address their common genetic background, established and suspected virulence-associated yersinial markers of the known virulent serobiotypes O:3/4 and O:8/1B have been determined and compared in the BT 1A strains (Tables 1–3). As expected, in addition to the pYV virulence plasmid, such markers of the virulent serobiotypes, like the Ail adhesin, the siderophore yersiniabactin (encoded as a part of the high pathogenicity island in BT 1B), the virulence-associated proteins VapC and VagC, and the enterotoxin YstA are absent from both 1A genomes (Table 1). Interestingly, no insecticidal toxin cluster genes have been found in both BT 1A isolates. These genes are described for serobiotype O:3/4 and other low-pathogenic serogroups and have been reported in 1A isolates (Tennant et al., 2005). However, a large number of 1A genes is shared (having limited, but still evident similarity) with either