Yersinia massiliensis sp. nov., isolated from fresh water

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Two bacterial organisms, 50640^T and 823, were isolated from fresh water in Marseilles, France, and were further identified as members of the genus *Yersinia* on the basis of their phenotypic characteristics and 16S rRNA gene sequencing. Their unique phenotypic profile differed from that of closely related species of *Yersinia bercovieri* and *Yersinia mollaretii* by exhibiting positive indole and inositol tests, and from that of *Yersinia frederiksenii* by lacking the ability to ferment L-rhamnose. A polyphasic approach, including almost complete 16S rRNA gene sequencing (1461 bp) and partial sequencing of *hsp60* (683 bp), *gyrB* (662 bp), *sodA* (624 bp) and *rpoB* (1049 bp) showed that isolates 50640^T and 823 exhibited 98.5, 93.5, 90.4, 92.4 and 96.6 % similarity with *Y. mollaretii*, 98.7, 93.0, 90.1, 89.1 and 96.2 % with *Y. bercovieri*, and 98.4, 93.2, 89.8, 88.9 and 95.2 % with *Y. frederiksenii*, respectively. Both isolates exhibited an identical 16S rRNA gene sequence and differed by one to five point mutations in housekeeping gene sequences. Phylogenetic reconstructions based on the combination of these four housekeeping genes indicated that the two isolates formed a unique branch supported by a bootstrap value of 93 %. Their unique phenotypic traits, 16S rRNA gene sequence, together with housekeeping gene sequences exhibiting <97 % similarity with closely related species, and phylogenetic analyses suggested that the two isolates represent a so far undescribed *Yersinia* species. The name *Yersinia massiliensis* sp. nov. is proposed for this new taxon (type strain 50640^T=CIP 109351^T=CCUG 53443^T; isolate 823=CIP 109352=CUG 53444).

At the time of writing, the genus *Yersinia* consists of 12 species, including *Yersinia pestis* (the plague agent), *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (the two species responsible for a complex clinical condition known as yersiniosis) (Perry & Fetherston, 1997; Bottone, 1999; Carniel & Mollaret, 1990), and nine *Y. enterocolitica*-like species, i.e. *Yersinia frederiksenii*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia bercovieri*, *Yersinia mollaretii*, *Yersinia rohdei*, *Yersinia ruckeri*, *Yersinia aldovae* (Sulakvelidze, 2000) and *Yersinia aleksiciae* (Sprague & Neubauer, 2005). Except for *Y. pestis*, *Yersinia* species are ubiquitous and have been found in soil, fresh water sources, animals, foods and sewage (Bercovier & Mollaret, 1984; Bottone, 1997; Sulakvelidze, 2000). They have been occasionally isolated from clinical specimens (Gray, 1995) and virulence factors have been

detected in several isolates of these species (Robins-Browne *et al.*, 1991; Sulakvelidze *et al.*, 1999; Sulakvelidze, 2000). Herein we have characterized two freshwater *Yersinia* isolates by extensive phenotypic and molecular methods and found them to exhibit unique characteristics, suggesting that they are representative of a hitherto undescribed *Yersinia* species.

Isolate 50640^T was isolated in a dialysis unit from the hospital water distribution system in Marseilles, France, and isolate 823 came from a well in another part of southern France. Water specimens were filtered through a 0.45 µm nitrocellulose filter membrane (Millipore) and incubated at 30 °C for 5 days on cefsulodin-irgasan-novobiocin agar (Becton Dickinson) (Schiemann, 1979). Strains *Y. mollaretii* CCUG 26331^T, *Y. bercovieri* CCUG 26329^T, *Y. frederiksenii* CCUG 11293^T, *Y. aldovae* CCUG 18770^T, *Y. intermedia* CCUG 11292^T, *Y. kristensenii* CCUG 11294^T, *Y. rohdei* CCUG 38833^T, *Y. ruckeri* CCUG 14190^T, *Y. enterocolitica* CCUG 11291^T, *Y. enterocolitica* CCUG 12369^T, *Y. enterocolitica* CCUG 7758 and *Y. enterocolitica* CCUG 8239A were obtained from the Culture Collection, University of Göteborg, Sweden, and investigated in parallel. Motility was checked by microscopic observation. Morphological properties were determined by

The GenBank/EMBL/DDBJ accession numbers for the gene sequences of isolates 50640^T and 823 are EF179119 and EF179120 (16S rRNA gene), EF173172 and EF173177 (*hsp60*), EF175588 and EF175591 (*gyrB*), EF175618 and EF175621 (*sodA*), and EF175599 and EF175600 (*rpoB*), respectively.

Phylogenetic trees based on *rpoB*, *hsp60*, *gyrB* and *sodA* gene sequences of isolates 50640^T and 823 and 11 *Yersinia* species are available with the online version of this paper.

Gram staining. The size and ultrastructure of the cells were determined by transmission electron microscopy. Colonies were harvested from trypticase soy medium (bioMérieux), pelleted by centrifugation and prefixed for 1 h at room temperature in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) containing 0.1 M sucrose. After washing overnight with the same buffer, the bacteria were fixed for 1 h at room temperature with 1 % osmium tetroxide in 0.1 M cacodylate buffer, dehydrated through increasing concentrations (25–100 %) of ethanol, and then embedded in Epon 812. Thin sections were cut and post-stained with a saturated solution of methanol uranyl acetate and lead citrate in water before examination on a Morgagni M 268D electron microscope (FEI France) at an operating voltage of 60 kV. Oxidase activity was detected by using a dimethyl-*p*-phenylenediamine oxalate disk (Sanofi Diagnostic Pasteur). Catalase activity was detected by emulsifying a colony in 3 % hydrogen peroxide and checking for the presence of microscopic bubbles. Mannitol-fermenting colonies were identified by using an API 20E strip (bioMérieux), a commercial identification system for *Enterobacteriaceae* (Neubauer *et al.*, 1998), according to the instructions of the manufacturer with the exception of incubation at 28 °C (Sharma *et al.*, 1990). API 20E strips were read after 24, 48 and 72 h incubation under a highly humidified atmosphere, and tentative identifications were obtained using the Analytical Profile Index, fourth edition (Archer *et al.*, 1987; Sharma *et al.*, 1990). API Coryne, Api NH and API 20A (bioMérieux) were used for further determination of pyrazinamidase, lipase, aesculin and salicin. Isolates were serotyped by slide agglutination with antisera against *Y. enterocolitica* O:3, O:8 and O:9 (Denka Seiken). Every test was done in triplicate on three separate days to ensure the reproducibility of the results.

After 24 h culture on MacConkey agar, DNA extraction, purification, degradation and G+C content determination by HPLC were performed as described by Mesbah *et al.* (1989), except that a Waters 625 LC system with a Waters 486 Tenable Absorbance Detector and a Water 746 Data Module (Millipore) were used. Three determinations were done. DNA extracted from colonies by using Qiagen columns (QIAamp tissue kit) was used as a template for PCR amplification and sequencing of the 16S rRNA gene using primers fd1/rp2 (Weisburg *et al.*, 1991). Four additional housekeeping genes, including *rpoB*, *hsp60*, *gyrB* and *sodA*, were amplified and sequenced. *rpoB* gene amplification and sequencing was performed as described by Mollet *et al.* (1997). Because of limited sequence availability of the remaining three genes for some of the *Yersinia* species in GenBank, a 683 bp fragment of the *hsp60* gene was amplified and sequenced using primers hsp60-498F (5'-GGAAAAAGTCGGTAAAGAAGGCG-3') and hsp60-1181R (5'-GCTTCTCTCTCTTTCATTTCAACTTC-3') designed after alignment of the *hsp60* gene of *Y. enterocolitica* Y-108c (GenBank accession no. X68526), *Y. pseudotuberculosis* IP32953 (NC006155, locus_tag YPTB0405) and *Y. pestis*

CO92 (NC003143, locus_tag YPO0351). A 662 bp *gyrB* gene fragment was amplified and sequenced by using primers *gyrB*-303F (5'-CGGTAAATTTGACGATAACTCC-3') and *gyrB*-965R (5'-ACAGCAATCAAGCCTTCACGGG-3') designed after alignment of the *gyrB* gene of *Y. enterocolitica* ATCC 2610 (AB084022), *Y. pseudotuberculosis* IP32953 (NC006155, locus_tag YPTB3940) and *Y. pestis* CO92 (NC003143, locus_tag YPO4094). A 624 bp *sodA* gene fragment was amplified and sequenced by using the primer pair *sodA*-1F (5'-ATGAGTTAYTCACTGCCAT-3') and *sodA*-624R (5'-TTACTTRGCTTGAGCGAA-3') designed after alignment of the *sodA* gene of *Y. enterocolitica* WA-314 (X96852), *Y. pseudotuberculosis* (NC006155, locus_tag YPTB3925) and *Y. pestis* CO92 (NC003143, locus_tag YPO4061). Products of sequencing reactions were recorded with an ABI Prism 3130 *xl* DNA sequencer following the standard protocol of the supplier (Perkin Elmer Applied Biosystems). The percentage similarity between the sequences was determined using the CLUSTAL W program supported by the PBIL website (<http://npsa-pbil.ibcp.fr/>). For phylogenetic analysis, sequences were trimmed in order to start and finish at the same nucleotide position for all the strains under study. Multisequence alignment was performed by using the CLUSTAL_X program, version 1.81 in the PHYLIP software package (Thompson *et al.*, 1994). Phylogenetic trees were obtained from DNA sequences by using the neighbour-joining method with Kimura's two-parameter distance correction model with 1,000 bootstrap replications in the MEGA version 3.1 software package (Kumar *et al.*, 2004). Phylogenetic trees based upon each gene sequence and one based on the combined gene sequences were then compared using the neighbour-joining and maximum-parsimony methods.

Isolates 50640^T and 823 were shown to be Gram-negative bacilli and were motile at 28 °C. The rods were 2.23 µm long and 0.85 µm wide, with one to two flagella of 6.75 µm (Fig. 1). Biochemical characteristics that differentiate isolates 50640^T and 823 from closely related *Yersinia* species are summarized in Table 1. Both isolates were catalase-positive and oxidase-negative. An API 20E strip inoculated with isolate 50640^T or 823 yielded a biochemical pattern indistinguishable from that of *Y. enterocolitica* coded as 1154723 in the API 20E system. Parallel inoculation of *Y. enterocolitica* CCUG 12369^T, *Y. frederiksenii* CCUG 11293^T and *Y. pseudotuberculosis* IP 32953 yielded codes of 1154723, 1354733 and 1014113, respectively, allowing very good identification of these species. However, the codes 1114523 for *Y. bercovieri* and 1114523 for *Y. mollaretii* identified these strains as *Y. enterocolitica* according to the API 20E coding system. Isolates 50640^T and 823 differed from *Y. mollaretii* CCUG 26331^T and *Y. bercovieri* CCUG 26329^T by exhibiting positive indole and inositol tests, and from *Y. frederiksenii* CCUG 11293^T by the lack of L-rhamnose fermentation and citrate utilization. After 48–72 h incubation, isolates 50640^T and 823 exhibited citrate utilization and weak acetoin production, resulting in a profile number of 1354723 that identified

Fig. 1. Electron micrograph of cells of isolate 50640^T. Bar, 2 µm.

them as *Y. enterocolitica* (52 % confidence) or *Y. frederiksenii* (48 % confidence). The two isolates were positive for salicin, aesculin and pyrazinamidase, but did not react with any of the tested *Yersinia* typing sera.

The DNA G+C content was 48.7 ± 0.6 mol%. The 1461 bp 16S rRNA gene sequences of isolates 50640^T and 823 were identical and shared 98.7 % similarity with *Y. bercovieri*, 98.5 % with *Y. mollaretii* and 98.4 % with *Y. frederiksenii* corresponding to 17, 20 and 21 bp differences, respectively (Table 2). The 16S rRNA gene sequence did not discriminate *Y. enterocolitica* CCUG 11291^T, *Y. enterocolitica* CCUG 12369^T and *Y. enterocolitica* CCUG 7758. As for the *hsp60*, *gyrB*, *sodA* and *rpoB* gene sequences,

isolates 50640^T and 823 shared 93.5, 90.4, 92.4 and 96.6 % similarity with the respective homologous sequences of *Y. mollaretii*, 93.0, 90.1, 89.1 and 96.3 % with *Y. bercovieri* and 93.2, 89.8, 88.9 and 95.3 % with *Y. frederiksenii* (Table 2). Comparison of sequences derived from isolates 50640^T and 823 yielded one point mutation in *gyrB*, two in *sodA*, four in *hsp60* and five in *rpoB*.

Phylogenetic trees were constructed based upon each gene sequence, 16S rRNA (1461 bp), *hsp60* (635 bp), *gyrB* (619 bp), *sodA* (624 bp) and *rpoB* (1049 bp), and by combining the four housekeeping gene sequences (about 3000 nt) determined for 11 *Yersinia* species in addition to isolates 50640^T and 823 (Figs 2 and 3). Bootstrap values >75 % in the 16S rRNA gene tree occurred for only 7/12 (58.3 %) nodes, too low to provide much confidence (Fig. 2). Likewise, *hsp60*-, *gyrB*-, *sodA*- and *rpoB*-based phylogenetic trees showed low bootstrap values with only 7/13 (51.8 %), 5/12 (41.7 %), 4/13 (30.8 %) and 8/13 (61.5 %) nodes >75 %, respectively (see supplementary Figs S1, S2, S3 and S4 available with the online version of this paper). In contrast, the combination of the four housekeeping gene sequences improved the bootstrap values with 12/13 (92.3 %) nodes >75 % ($P < 0.05$) (Fig. 3). This analysis also revealed that isolates 50640^T and 823 formed a unique line within the genus *Yersinia*. A bootstrap value of 93 % in the neighbour-joining tree supported the fork separating isolates 50640^T and 823 from *Y. mollaretii* and *Y. bercovieri*, giving a high level of confidence to this analysis (Fig. 3). Parsimony and maximum-likelihood methods confirmed that isolates 50640^T and 823 formed a lineage that was clearly different from closely related species and quite distant from other recognized *Yersinia* species. In most, but not all cases, the four housekeeping genes and the 16S rRNA gene sequence trees correlated well with each other, and agreed with a separate species status for isolates 50640^T and 823 (Figs 2 and 3).

Two isolates, 50640^T and 823, of biochemically atypical *Yersinia* were recovered from haemodialysis unit water and well water. An API 20E strip misidentified these isolates as

Table 1. Biochemical characteristics that distinguish isolates 50640^T and 823 from closely related species

Other tests including catalase, oxidase, β -galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H₂S, urease, tryptophan deaminase, gelatinase, glucose, mannitol, sorbitol, sucrose, melibiose, amygdalin, arabinose, salicin, pyrazinamidase and aesculin were not discriminant. +, Positive; –, negative; $\pm$, weakly positive after 48 h.

Characteristic	Isolate 50640 ^T CCUG 53443 ^T	Isolate 823 CCUG 53444	<i>Y. frederiksenii</i> CCUG 11293 ^T	<i>Y. mollaretii</i> CCUG 26331 ^T	<i>Y. bercovieri</i> CCUG 26329 ^T
Simmons citrate	–	–	+	–	–
Indole	+	+	+	–	–
Voges–Proskauer*	$\pm$	$\pm$	$\pm$	–	–
Inositol	+	+	+	–	–
Rhamnose	–	–	+	–	–
API Profile number	1154723	1154723	1354733	1114523	1114523

*Determined after 3 days incubation at 28 °C.

Table 2. Gene sequence similarities of isolates 50640^T and 823 with 11 *Yersinia* species, and similarity of *Y. mollaretii* and *Y. bercovieri*, and *Y. aldovae* and *Y. intermedia* with respect to 16S rRNA and four housekeeping genes

Species	16S rRNA (1461 bp)	<i>hsp60</i> (635 bp)	<i>gyrB</i> (619 bp)	<i>sodA</i> (624 bp)	<i>rpoB</i> (1,049 bp)
<i>Y. frederiksenii</i> CCUG 11293 ^T	98.4	93.2	89.8	88.9	95.2
<i>Y. bercovieri</i> CCUG 26329 ^T	98.7	93.0	90.1	89.1	96.2
<i>Y. mollaretii</i> CCUG 26331 ^T	98.5	93.5	90.4	92.4	96.6
<i>Y. aldovae</i> CCUG 18770 ^T	98.2	92.1	88.2	88.6	94.3
<i>Y. intermedia</i> CCUG 11292 ^T	98.6	93.0	89.1	88.4	94.3
<i>Y. kristensenii</i> CCUG 11294 ^T	98.1	93.0	90.4	88.9	94.0
<i>Y. rohdei</i> CCUG 38833 ^T	98.2	90.8	90.4	84.4	95.6
<i>Y. ruckeri</i> CCUG 14190 ^T	98.4	88.6	89.6	22.7	94.3
<i>Y. enterocolitica</i> CCUG 7758	97.8	92.7	90.7	88.7	95.0
<i>Y. enterocolitica</i> CCUG 8239A	98.3	93.0	90.4	89.2	95.1
<i>Y. enterocolitica</i> CCUG 11291 ^T	97.8	92.5	90.7	88.3	95.1
<i>Y. enterocolitica</i> CCUG 12369 ^T	97.8	92.5	90.7	88.3	95.0
<i>Y. pestis</i> CO92	98.5	91.6	89.3	88.7	93.5
<i>Y. pseudotuberculosis</i> IP32953	98.5	91.8	89.4	88.6	93.5
<i>Y. mollaretii</i> CCUG 26331 ^T vs <i>Y. bercovieri</i> CCUG 26329 ^T	99.3	94.9	92.5	91.3	97.9
<i>Y. aldovae</i> CCUG 18770 ^T vs <i>Y. intermedia</i> CCUG 11292 ^T	99.5	93.5	91.7	92.9	96.9

Y. enterocolitica. However, the two isolates were positive for salicin, aesculin, pyrazinamidase, lipase, xylose, trehalose, indole and the Voges–Proskauer test, a pattern in agreement with the recent description of some *Y. enterocolitica*-like species (Hallanvuo *et al.*, 2006). We observed a 1.3 % divergence in the 16S rRNA gene sequence of isolates 50640^T and 823 with that of *Y. bercovieri*, 1.5 % with that of *Y. mollaretii* and 1.6 % with that of *Y. frederiksenii*. These levels were below the 1.3 % threshold proposed by Stackebrandt & Ebers (2006) to delineate bacterial species by using 16S rRNA gene sequencing, thus firmly suggesting that the isolates herein studied belong to a new species in agreement with their unique phenotypic profile.

We further characterized isolates 50640^T and 823 by sequencing four housekeeping genes to fulfil the recommendations of the ad-hoc committee for the re-evaluation of the definition of the bacterial species (Stackebrandt *et al.*, 2002) and as recently suggested for *Yersinia* isolates speciation (Kotetishvili *et al.*, 2005). These four housekeeping genes are spaced well apart on the genome and could potentially reflect the evolution of the whole genome (Thomson *et al.*, 2006). The topologies of the four housekeeping-based phylogenetic trees and the 16S rRNA gene-based phylogenetic tree were quite similar. However, the four housekeeping genes were more consistent with the biochemical species designation than the 16S rRNA gene

Fig. 2. Phylogenetic tree based on the 16S rRNA gene sequences of isolates 50640^T and 823 and 11 *Yersinia* species prepared by using the neighbour-joining method and Kimura's two-parameter distance correction model. GenBank accession numbers are given in parentheses. The support of each branch, as determined from 100 bootstrap samples, is indicated by the value (%) at each node. Only bootstrap values ≥ 75 % are indicated. The scale bar represents a 0.2 % difference in nucleotide sequence.

Fig. 3. Phylogenetic tree based on concatenated *rpoB*, *hsp60*, *gyrB* and *sodA* gene sequences of isolates 50640^T and 823 and 11 *Yersinia* species prepared by using the neighbour-joining method and Kimura's two-parameter distance correction model. The support of each branch, as determined from 100 bootstrap samples, is indicated by the value (%) at each node. Only bootstrap values $\geq 75\%$ are indicated. The scale bar represents a 2% difference in nucleotide sequence.

analysis, i.e. *Y. mollaretii* and *Y. bercovieri*, which exhibited a similar biochemical profile, were in the same branch in the four housekeeping gene trees (Fig. 3) whereas they were in separate branches in the 16S rRNA gene tree (Fig. 2). The four housekeeping genes yielded bootstrap values higher than those derived from the 16S rRNA gene. These data indicate that the 16S rRNA gene sequence alone cannot resolve the phylogenetic relationships between all currently recognized *Yersinia* species.

By combining the four housekeeping genes studied herein, we found that *Y. mollaretii* and *Y. bercovieri* on one hand, and *Y. aldovae* and *Y. intermedia* on the other were closely related. In the *rpoB*, *gyrB*, *hsp60* and *sodA* gene sequences, similarity levels between *Y. mollaretii* and *Y. bercovieri* were 97.9, 92.5, 94.9 and 91.3%, and 96.9, 91.7, 93.5 and 92.9% between *Y. aldovae* and *Y. intermedia*, respectively (Table 2). Isolates 50640^T and 823 showed less similarity with respect to the four housekeeping genes with all other *Yersinia* species (Table 2). Although there are no validated cut-off values for *Yersinia* species delineation, we observed that similarity values of $<98.0\%$ for *rpoB*, $<92.5\%$ for *gyrB*, $<95.0\%$ for *hsp60* and $<93.0\%$ for *sodA* effectively delineated the currently recognized species in the *Yersinia* genus. These values supported previous DNA–DNA hybridization data for the genus (Bottone, 1997). As for the *rpoB* gene, these cut-off values agree with the similarity

value (97.6% over 1049 bp) that delineates two other enteric species, *Klebsiella variicola* and *Klebsiella pneumoniae*; these species share 99.3% 16S rRNA gene sequence similarity (Rosenblueth *et al.*, 2004). The same strategy, based on this short *rpoB* gene portion, has been used to delineate *Klebsiella singaporensis* from *K. pneumoniae*, two species which share 99.3% 16S rRNA gene sequence similarity and 97.5% *rpoB* gene sequence similarity (Li *et al.*, 2004). Based on a 512 bp portion of the *rpoB* gene sequence reported for *Enterobacteriaceae* species (Mollet *et al.*, 1997), these authors found 97.7–98.8% similarity among representative species of three genetic groups, i.e. *Klebsiella*, *Escherichia* and *Salmonella*. We found that the interspecies similarity in this region for the 11 *Yersinia* species examined in this study varied from 93.5% (*Y. pestis* and *Y. enterocolitica*) to 98.8% (*Y. kristensenii* and *Y. enterocolitica*). Also, our data showed that isolates 50640^T and 823 shared 96.6% similarity with *Y. mollaretii*, 96.2% with *Y. bercovieri*, and 95.2% with *Y. frederiksenii*. This analysis further supported that isolates 50640^T and 823 formed a novel species within the genus *Yersinia*.

Comparative 16S rRNA gene sequence analysis revealed that isolates 50640^T and 823 shared 100% sequence similarity with non-typable *Yersinia* isolate 11A recovered from raw milk samples in Queensland, Australia (Ibrahim *et al.*, 1997). The dendrogram of relationships based on the 16S rRNA gene sequence indicated that the atypical isolate 11A formed an individual line of descent distinct from *Y. frederiksenii* (Ibrahim *et al.*, 1997). Although 16S rRNA gene sequence identity does not necessarily indicate close relatedness, this *Yersinia* isolate also exhibited a similar biochemical phenotype to isolates 50640^T and 823. It yielded only 41% DNA–DNA relatedness to the *Y. frederiksenii* type strain (Ibrahim *et al.*, 1997) and has been tentatively assigned to *Y. frederiksenii* hybridization group 2 (unnamed genospecies 2). Isolate 11A, however, was not available for this study and the question of its taxonomic status remains open, pending further molecular comparisons with isolates 50640^T and 823.

Original phenotypic traits and molecular analysis, including sequence analyses of the 16S rRNA, *hsp60*, *sodA*, *gyrB* and *rpoB* genes, identified isolates 50640^T and 823 as belonging to a novel *Yersinia* species, for which the name *Yersinia massiliensis* is proposed.

Description of *Yersinia massiliensis* sp. nov.

Yersinia massiliensis (mas.si.li.en'sis. L. fem. adj. *massilien-sis* pertaining to Massilia, the ancient Roman name of Marseille, France, where the type strain was isolated).

Freshwater-living Gram-negative, coccoid rod, motile with one to two flagella of 6.75 μm . Colonies appeared on 5% blood sheep agar after 24 h incubation. The colonies are circular and convex. Growth occurs at 28 and 37 °C. Cells are catalase-positive and oxidase-negative. Positive reaction for urease, indole, ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase and tryptophan deaminase,

and delayed citrate utilization. Glucose, mannitol, inositol, sorbitol, sucrose, amygdale and arabinose are fermented, but rhamnose and melobiose are not. Gelatin was not hydrolysed. Voges–Proskauer test was negative after 24 h incubation and weakly positive on day 3. The G+C content was 49.3 mol%. 16S rRNA, *hsp60*, *gyrB*, *sodA* and *rpoB* gene sequences share 98.5, 93.5, 90.4, 92.4 and 96.6 % similarity with *Y. mollaretii*, 98.7, 93.0, 90.1, 89.1 and 96.2 % similarity with *Y. bercovieri* and 98.4, 93.2, 89.8, 88.9 and 95.2 % similarity with *Y. frederiksenii*, respectively.

The type strain, isolated in a dialysis unit from the hospital water distribution system in Marseilles, France, is 50640^T (=CIP 109351^T=CCUG 53443^T). Strain 823 (=CIP 109352=CCUG 53444) was isolated from well water in southern France.

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