Emended description of *Yersinia massiliensis*

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The bacterial genus *Yersinia* belongs to the family *Enterobacteriaceae* and comprises 15 species. Species of the genus *Yersinia* are usually identified by their phenotypic characteristics. Thus, it is essential to establish a complete phenotypic classification for all species of the genus *Yersinia*. The species *Yersinia massiliensis* was proposed in 2008, based on 16S rRNA, *gyrB*, *hsp60*, *rpoB* and *sodA* gene sequences and some distinguishing phenotypic characteristics. In this study, four *Yersinia* strains classified as *Y. massiliensis* based on the sequencing of the loci mentioned above were subjected to a more detailed phenotypic characterization. This characterization revealed differences in the results of four tests previously reported as diagnostic for *Y. massiliensis* and the results of 18 additional tests provided new information about the biochemical diversity of this species. In the light of the results of the phenotypic characteristics of the four strains of *Y. massiliensis*, an emended description of *Y. massiliensis* is presented.

The bacterial genus *Yersinia* belongs to the family *Enterobacteriaceae* and comprises 15 recognized species. *Yersinia pestis*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* are associated with human and animal diseases. *Yersinia ruckeri* is a fish pathogen and all the other species are considered to be mainly non-pathogenic and environmental isolates (Wanger, 2007). The newest members of the genus are *Yersinia aleksiciae* (Sprague & Neubauer, 2005), *Yersinia similis* (Sprague *et al.*, 2008), *Yersinia massiliensis* (Merhej *et al.*, 2008) and *Yersinia entomophaga* (Hurst *et al.*, 2011).

Four *Y. massiliensis* strains selected from the collection of the Brazilian Reference Center on *Yersinia* spp. other than *Y. pestis* shared many of the phenotypic characteristics with strain CCUG 53443^T, the type strain of *Y. massiliensis*. However, they differed from this type strain in the results of four biochemical tests used to characterize this species, as described in Merhej *et al.* (2008). Further information on the four *Y. massiliensis* strains used in this study is given in Table 1. It is important to highlight that these *Yersinia* strains had been isolated from environmental and food sources and had not been implicated in human disease.

The four strains, FCF 216, FCF 465, FCF 457 and FCF 494, were assigned as belonging to the species *Y. massiliensis* based on the sequences of five conserved loci (16S rRNA,

gyrB, *hsp60*, *rpoB* and *sodA* gene sequences). Internal fragments of these loci were amplified for the four strains and were compared with sequences of the same loci for other species of the genus *Yersinia* that had already been deposited in GenBank (Kotetishvili *et al.*, 2005; Sprague & Neubauer, 2005; Merhej *et al.*, 2008; Sprague *et al.*, 2008; Hurst *et al.*, 2011). The sequence and phylogenetic analyses were performed as described by Souza *et al.* (2010). Phylogenetic trees constructed from the 16S rRNA gene sequences and also from a 'super locus' consisting of the concatenated *gyrB*, *hsp60*, *rpoB* and *sodA* sequences for each strain are available as supplementary data with the online version of this paper (Supplementary Figs S1 and S2 in IJSEM Online).

In order to elucidate the phenotypic diversity of *Y. massiliensis* species, strains FCF 216, FCF 465, FCF 457, FCF 494 and CCUG 53443^T were subjected to a battery of phenotypic tests. The strains were reisolated on MacConkey agar (Acumedia). They were subjected to the phenotypic tests shown in Table 2. Carbohydrate fermentation and citrate utilization were carried out at 28 °C for 7 days. The other tests were performed at 28 °C for 48 h. The motility assay was conducted at 28 °C and 37 °C for 48 h. Oxidase activity was detected by using a dimethyl-*p*-phenylenediamine oxalate disk (Newprov). Catalase activity was detected by emulsifying a colony in 3 % hydrogen peroxide and checking for the presence of bubbles. Other tests, such as citrate utilization (Acumedia), phenylalanine deaminase (Difco) and urea hydrolysis (Acumedia) were performed according to the manufacturers' instructions.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA, *gyrB*, *hsp60*, *rpoB* and *sodA* gene sequences described in this study are GQ421616–GQ421635.

Supplementary figures are available with the online version of this paper.

Table 1. Summary of some characteristics of the four *Y. massiliensis* strains used in this study

Strain	Source	Country	Year	Serogroup*–phage typed†
<i>Y. massiliensis</i> FCF 216	Water	Brazil	1984	O:10-Xz
<i>Y. massiliensis</i> FCF 465	Water	Argentina	1990	O:10, 34-Xo
<i>Y. massiliensis</i> FCF 457	Pasteurized milk	Brazil	1990	O:22-Xo
<i>Y. massiliensis</i> FCF 494	Lettuce	Brazil	1991	O:10, 34-Xo

*According to Wauters *et al.* (1991).†According to Nicolle *et al.* (1976).

Carbohydrate fermentation was conducted as described in MacFaddin (2000) using each carbohydrate at a concentration of 0.5 %, with the exception of methyl α -D-glucoside, which was used at 1.0 %. Gas from glucose and H₂S production were observed in triple-sugar iron agar (HiMedia Laboratories), according to the manufacturer's instructions. The other tests were all prepared in house. Nitrate reduction, Voges–Proskauer reaction, indole production, ornithine decarboxylase, lysine decarboxylase, arginine dihydrolase and motility tests were prepared as reported in MacFaddin (2000). Salicin fermentation and

aesculin hydrolysis were performed as reported in Farmer *et al.* (1992). Gelatin hydrolysis and lipase were carried out according to the methods of Dempsey & Kitting (1987). Mucate utilization and pyrazinamidase production were conducted as described by the US Food and Drug Administration (2010) and Kandolo & Wauters (1985), respectively.

In order to perform a more detailed phenotypic characterization of the species *Y. massiliensis*, the following tests were performed in addition to those previously conducted by Merhej *et al.* (2008): lactose, raffinose, methyl α -D-glucoside, cellobiose, D-trehalose, L-sorbose, maltose, L-fucose, salicin and D-xylose fermentation, plus gas from glucose, H₂S production, mucate utilization, nitrate reduction, pyrazinamidase production, lipase, aesculin hydrolysis and motility at 37 °C.

Table 2. Comparison of phenotypic characteristics between *Y. massiliensis* strains FCF 216, FCF 465, FCF 457, FCF 494 and *Y. massiliensis* CCUG 53443^T

Data for strain CCUG 53443^T are from Merhej *et al.* (2008). Tests for L-rhamnose, melibiose, methyl α -D-glucoside* fermentation, oxidase, gas from glucose*, Voges–Proskauer, H₂S production*, lipase*, gelatin hydrolysis and motility at 37 °C* were negative for all five strains. Tests for cellobiose*, sucrose, trehalose*, L-sorbose*, D-sorbitol, maltose*, L-fucose*, salicin*, inositol, L-arabinose, D-xylose*, D-mannitol fermentation, mucate utilization*, nitrate reduction*, indole production, ornithine decarboxylase, pyrazinamidase production*, urea hydrolysis, aesculin hydrolysis*, catalase and motility at 28 °C were positive for all five strains. +, Positive; –, negative; ND, not done. For carbohydrate fermentation and citrate utilization tests, incubation was performed at 28 °C for 7 days. For the remaining tests, incubation was conducted at 28 °C for 48 h. *Additional tests performed in this study.

Characteristic	CCUG 53443 ^T	FCF 216	FCF 465	FCF 457	FCF 494
Lactose fermentation	ND†	–	+	+	–
Raffinose fermentation	ND†	–	–	+	–
Citrate utilization	+	–	–	–	+
Lysine decarboxylase	+†	–	–	–	–
Arginine dihydrolase	+†	–	–	–	–
Phenylalanine deaminase	+†	–	–	–	–

†Data shown are from Merhej *et al.* (2008). These tests were negative under our conditions of incubation at 28 °C for 48 h.

The results of the tests on strains FCF 216, FCF 465, FCF 457 and FCF 494 are summarized in Table 2 and are compared with the phenotypic characteristics of *Y. massiliensis* CCUG 53443^T as proposed by Merhej *et al.* (2008). The results of three biochemical tests reported by Merhej *et al.* (2008) for *Y. massiliensis* were significantly different from those obtained for the *Y. massiliensis* strains in this study. *Y. massiliensis* strains CCUG 53443^T and CCUG 53444 were reported as positive in tests for lysine decarboxylase, arginine dihydrolase and phenylalanine deaminase by Merhej *et al.* (2008), but the four strains from our collection gave negative results. However, under our conditions of incubation at 28 °C for 48 h, *Y. massiliensis* CCUG 53443^T showed negative results for the lysine decarboxylase, arginine dihydrolase and phenylalanine deaminase reactions.

As described by Bottone *et al.* (2005), all species of the genus *Yersinia* are lysine decarboxylase-, phenylalanine deaminase- and arginine dihydrolase-negative. However, Bottone *et al.* (2005) and Sprague & Neubauer (2005) have reported lysine decarboxylase activity in *Y. aleksiciae* and in some strains of *Y. ruckeri*, *Y. kristensenii* and *Y. intermedia*. Farmer *et al.* (1985) reported that a small number of *Y. ruckeri* strains were arginine dihydrolase-positive.

In this study, variable results were also found for citrate utilization. Specifically, three of the *Y. massiliensis* strains (FCF 216, FCF 465 and FCF 457) gave negative results for

citrate utilization and only strain FCF 494 was able to utilize citrate. Citrate utilization was reported as positive for *Y. massiliensis* strains CCUG 53443^T and CCUG 53444 by Merhej *et al.* (2008).

It is suggested that the phenotypic differentiation of *Y. massiliensis* from other species of the genus *Yersinia* should not just be based on the phenotypic tests proposed by Bockemühl & Wong (2003), Ewing *et al.* (1978), Sprague & Neubauer (2005), Sprague *et al.* (2008) and Hurst *et al.* (2011). *Y. enterocolitica*, *Y. kristensenii*, *Y. aleksiciae*, *Y. rohdei* and *Y. mollaretii* differ from *Y. massiliensis* by the result of only a single test. Thus, it could be possible to identify these species incorrectly. To avoid this problem, we suggest adding aesculin hydrolysis and mucate utilization to the phenotypic identification of species of the genus *Yersinia*, as presented in Table 3. With the addition of these two tests, it would be possible to differentiate all of the species of the genus *Yersinia* on the basis of phenotypic characteristics, with the exception of *Y. aleksiciae*, which cannot be separated from *Y. kristensenii* based solely on phenotypic tests.

The results of the biochemical tests presented in Table 2 add new information about the biochemical diversity of *Y. massiliensis* strains and contribute to the further characterization of this species. Based on our results, a more detailed and broader description of the species *Y. massiliensis* is

proposed and an emended description of this species is presented.

Emended description of *Yersinia massiliensis* Merhej *et al.* 2008

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

Ferments cellobiose, trehalose, L-sorbose, maltose, L-fucose, salicin and D-xylose, but does not ferment methyl α -D-glucoside. Raffinose and lactose fermentation reactions are variable. Positive reactions are obtained in tests for mucate utilization, nitrate reduction, pyrazinamidase production and aesculin hydrolysis. Motility at 37 °C, gas production from glucose, lipase and H₂S production are negative. Citrate utilization is variable after incubation at 28 °C for 7 days. Arginine dihydrolase, lysine decarboxylase and phenylalanine deaminase activities are negative after incubation at 28 °C for 48 h. Using API 20E strips (bioMérieux), positive results are reported for citrate utilization, arginine dihydrolase, lysine decarboxylase and phenylalanine deaminase after 24, 48 and 72 h at 28 °C (Merhej *et al.*, 2008).

The type strain is CCUG 53443^T (=CIP 109351^T). This species has been isolated from the environment and food.

Table 3. Phenotypic differentiation of species of the genus *Yersinia*

Taxa: 1, *Y. pestis*; 2, *Y. pseudotuberculosis*; 3, *Y. similis*; 4, *Y. enterocolitica*; 5, *Y. intermedia*; 6, *Y. frederiksenii*; 7, *Y. kristensenii*; 8, *Y. aleksiciae*; 9, *Y. aldovae*; 10, *Y. rohdei*; 11, *Y. mollaretii*; 12, *Y. bercovieri*; 13, *Y. ruckeri*; 14, *Y. entomophaga*; 15, *Y. massiliensis*. Data are from Bockemühl & Wong (2003), Ewing *et al.* (1978), Farmer *et al.* (1985), Sprague & Neubauer (2005), Sprague *et al.* (2008) and Hurst *et al.* (2011) with additional information for *Y. massiliensis* based on the results from this study. Incubation was conducted at 28 °C for 7 days for carbohydrate fermentation and at 28 °C for 48 h for the other tests. +, $\geq 90\%$ strains positive; –, $\geq 90\%$ strains negative; v, variable, 11–89% of strains positive; ND, not done.

Characteristic	1	2	3	4	5	6	7	8*	9	10	11	12	13	14	15
Motility	–	+	+	+	+	+	+	+	+	+	+	+	v	+	+
Urease production	–	+	+	+	+	+	+	+	+	v	+	+	–	–	+
Voges–Proskauer reaction	–	–	–	+	+	+	–	–	+	–	–	–	–	ND	–
Indole production	–	–	–	v	+	+	v	v	–	–	–	–	–	–	+
Citrate utilization	–	–	–	–	+	v	–	–	v	+	–	–	+	+	v
Ornithine decarboxylase	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
Aesculin hydrolysis	v	+	+	v	+	v	–	–	–	–	–	v	–	–	+
Mucate utilization	–	–	–	–	–	–	–	ND	–	–	–	–	–	ND	+
Fermentation of:															
Cellobiose	–	–	–	+	+	+	+	+	–	+	+	+	–	+	+
Melibiose	v	+	–	–	+	–	–	–	–	v	–	–	–	+	–
Raffinose	–	v	–	–	+	–	–	–	–	v	–	–	–	+	v
L-Rhamnose	–	+	+	–	+	+	–	–	+	–	–	–	–	–	–
D-Sorbitol	–	–	–	v	+	+	+	+	+	+	+	+	–	–	+
L-Sorbose	–	–	–	v	+	+	+	v	–	+	+	–	–	–	+
Sucrose	–	–	–	+	+	+	–	–	–	+	+	+	–	+	+

**Y. aleksiciae* cannot be separated from *Y. kristensenii* based solely on phenotypic tests.

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