Yersinia pekkanenii sp. nov.

Anna Murros-Kontinen,¹ Per Johansson,¹ Taina Niskanen,^{1,2}
Maria Fredriksson-Ahomaa,¹ Hannu Korkeala¹ and Johanna Björkroth¹

Correspondence

Johanna Björkroth

johanna.bjorkroth@helsinki.fi

¹Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland

²Finnish Food Safety Authority Evira, Hygiene Unit, Helsinki, Finland

The taxonomic position of three strains from water, soil and lettuce samples was studied by using a polyphasic taxonomic approach. The strains were reported to lack the virulence-encoding genes *inv* and *virF* in a previous study. Controversially, API 20 E and some other phenotypic tests suggested that the strains belong to *Yersinia pseudotuberculosis*, which prompted this polyphasic taxonomic study. In both the phylogenetic analyses of four housekeeping genes (*glnA*, *gyrB*, *recA* and *HSP60*) and numerical analyses of *HindIII* and *EcoRI* ribopatterns, the strains formed a separate group within the genus *Yersinia*. Analysis of the 16S rRNA gene sequences showed that the strains were related to *Yersinia aldovae* and *Yersinia mollaretii*, but DNA–DNA hybridization analysis differentiated them from these species. Based on the results of the phylogenetic and DNA–DNA hybridization analyses, a novel species, *Yersinia pekkanenii* sp. nov., is proposed. The type strain is ÅYV7.1KOH2^T (=DSM 22769^T =LMG 25369^T).

This manuscript focuses on three strains: Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T, originating from water, soil and lettuce samples, respectively. API 20 E (bioMérieux) and some other phenotypic tests suggested that the strains belong to *Yersinia pseudotuberculosis*. This was considered controversial because, among others, the study of Niskanen *et al.* (2009) showed that the strains lacked the virulence-encoding genes *inv* and *virF*. In addition, their *NotI* digests, 16S–23S rRNA gene *HindIII* and *EcoRI* RFLP (ribotype) patterns and the O-antigen gene cluster were not typical for *Y. pseudotuberculosis*. In this study, multilocus sequence analysis of the 16S rRNA gene and four housekeeping genes, DNA G + C content determination and DNA–DNA hybridization tests clarified the taxonomic position of strains Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T. Additional phenotypic tests and *EcoRI* and *HindIII* ribotyping were also done. The *glnA*, *gyrB*, *recA* and *HSP60* genes, which had previously been used in a multilocus sequence typing study for the genus *Yersinia*, were chosen for the housekeeping-gene analysis because they provided the largest set of sequences for comparison. Two of these genes, *glnA* and *recA*, were used by Merhej *et al.* (2008) while describing the species *Yersinia massiliensis*.

Morphological properties of the strains were determined by Gram staining and transmission electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *glnA*, *gyrB*, *recA* and *HSP60* gene sequences of strains Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T are GQ451988–GQ452002.

A supplementary table and four supplementary figures are available with the online version of this paper.

Oxidase activity was tested with oxidase strips and catalase activity with 3 % hydrogen peroxide. Biochemical characterization was done using the API 20 E and API 50 CH strips according to the instructions of the manufacturer (bioMérieux). The incubation temperature used was 28 °C and the results were read after 24, 48 and 72 h. Each test was done at least twice. The motility test was performed at 28 and 37 °C using Motility Test and Maintenance and Motility Test media (Atlas & Snyder, 2006).

DNA from strains Å125KOH2, ÅYV25K1, ÅYV7.1KOH2^T, *Yersinia aldovae* CIP 103162^T and *Yersinia mollaretii* DSM 18520^T was extracted by the guanidium thiocyanate method described by Pitcher *et al.* (1989) with the addition of 0.6 mg proteinase K ml^{−1} to the cell suspension buffer. The *glnA*, *gyrB*, *recA* and *HSP60* genes were amplified using primers and amplification conditions described by Kotetishvili *et al.* (2005) with the exception of *gyrB* (the annealing temperature was raised to 62 °C). The 16S rRNA gene was amplified with universal primers F19–38 and R1541–1522 (Stackebrandt & Liesack, 1993). The PCR cycle for the 16S rRNA gene comprised denaturation at 98 °C for 2 min, followed by 30 amplification cycles (93 °C for 1 min, 54 °C for 2 min and 72 °C for 2.5 min) and final elongation at 72 °C for 8 min. Each PCR mixture consisted of 200 µM dNTPs, 0.02 IU DyNAZyme II polymerase µl^{−1} (Thermo Fisher Scientific Inc.) and 0.5 µM each primer. PCR products were purified by the Millipore MultiScreen PCR 96 system. Bidirectional sequencing of the PCR products was done with the same primers as were used for amplification, using an Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing kit

and an ABI 3130XL Genetic Analyzer. Sequences were aligned and consensus sequences were created using BioNumerics 5.10 software (Applied Maths). 16S rRNA, *glnA*, *gyrB*, *recA* and *HSP60* gene sequences of strains Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T were deposited in GenBank with accession numbers GQ451988–GQ452002. Supplementary Table S1 (available in IJSEM Online) presents the GenBank accession numbers of *glnA*, *gyrB*, *HSP60* and *recA* gene sequences for type and reference strains used in this study. Maximum-parsimony and neighbour-joining trees with the Jukes–Cantor correction and bootstrap analysis (1000 replicates) were constructed using BioNumerics 5.10 software. Phylogenetic trees were constructed using the 16S rRNA gene (1424 bp) and housekeeping genes *glnA* (494 bp), *gyrB* (471 bp), *recA* (483 bp) and *HSP60* (486 bp). Concatenated trees were constructed by combining *glnA*, *gyrB*, *recA* and *HSP60* gene sequences. *Serratia proteamaculans* was used as an outgroup.

Ribotyping procedures and numerical analyses of the patterns were done according to Niskanen *et al.* (2009). Supplementary Fig. S1 (available in IJSEM Online) presents *Hind*III and *Eco*RI ribopatterns of strains Å125KOH2,

ÅYV25K1 and ÅYV7.1KOH2^T and comparison with those of type strains of *Yersinia* species.

The DNA G+C content was determined as described by Xu *et al.* (2000). Melting-point curves were determined with a LightCycler instrument (Roche) using SYBR Green I dye (Roche); 1× SSC was used to obtain sharp melting curves. *Y. aldovae* CIP 103162^T was used as the reference organism and *Y. mollaretii* DSM 18520^T was used as the control.

DNA–DNA hybridization tests were done with strains ÅYV7.1KOH2^T, ÅYV25K1, *Y. aldovae* CIP 103162^T and *Y. mollaretii* DSM 18520^T according to Jakava-Viljanen *et al.* (2008). Each measurement was repeated three times.

Strains Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T were Gram-negative rods. The length of the cells was 1–3 µm (Supplementary Fig. S2) after 24 h incubation on blood agar at 30 °C. Colonies were circular, convex and of pinpoint size after 24 h on blood agar at 30 °C. Cells were non-motile at 28 and 37 °C in motility tests and under microscopic observation, oxidase-negative and catalase-positive. Biochemical characteristics of the strains are presented in Table 1 and are compared with those of other

Table 1. Biochemical characteristics of *Yersinia pekkanenii* sp. nov. compared with those of type strains of other *Yersinia* species

Type strains: 1, *Y. pekkanenii* sp. nov.; 2, *Y. pseudotuberculosis*; 3, *Y. aldovae*; 4, *Y. mollaretii*; 5, *Y. bercovieri*; 6, *Y. intermedia*; 7, *Y. rohdei*; 8, *Y. enterocolitica* subsp. *enterocolitica*; 9, *Y. enterocolitica* subsp. *paleoartica*; 10, *Y. aleksiciae*; 11, *Y. frederiksenii*; 12, *Y. kristensenii*; 13, *Y. pestis* (Sprague *et al.*, 2008); 14, *Y. ruckeri*; 15, *Y. similis*; 16, *Y. massiliensis*. All data are from this study unless otherwise indicated. Results are presented after 48 h incubation: +, positive; –, negative; (+), weakly positive. All strains were positive for utilization of trehalose and glucose and negative for utilization of adonitol.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Utilization of:																
D-Xylose	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	+
Sorbose	–	–	–	+	–	+	–	+	+	–	+	+	–	–	–	+
Aesculin	–	+	–	(+)	–	+	–	–	–	–	+	–	+	–	+	+
Salicin	–	+	–	(+)	(+)	+	(+)	–	–	(+)	+	+	–	–	(+)	+
Cellobiose	+	–	–	+	+	+	+	+	+	+	+	+	–	–	–	+
Lactose	(+)	–	–	(+)	–	–	(+)	–	–	–	–	+	–	–	–	–
Raffinose	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–
Glycerol	–	(+)	+	+	(+)	+	+	+	+	+	+	+	–	+	+	+
Citrate	–	–	+	+	–	+	+	–	–	–	+	–	–	–	+	+
Inositol	–	–	+	(+)	–	+	–	(+)	+	(+)	+	(+)	–	–	–	+
Sorbitol	–	–	+	+	+	+	+	+	+	+	+	+	–	–	–	+
Melibiose	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–
Rhamnose	–	(+)	+	–	–	+	–	–	–	–	+	–	–	–	+	–
ONPG	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+
Sucrose	–	–	–	+	+	+	+	+	+	–	+	–	–	–	–	(+)
L-Arabinose	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+	+
Urea hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+
Lysine decarboxylase	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Ornithine decarboxylase	–	–	+	+	+	+	+	+	+	+	+	+	–	+	–	+
Voges–Proskauer reaction	–	–	+	–	–	+	–	–	–	–	+	–	–	–	–	–
Indole production	–	–	–	–	–	+	–	+	–	–	+	+	–	–	–	+

Yersinia species. The API 20 E (bioMérieux) profile obtained for strains Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T was 1 0 1 4 1 0 3 (98.4 % identity with *Y. pseudotuberculosis*). As reported previously (Niskanen *et al.*, 2009), these strains cannot be differentiated from *Y. pseudotuberculosis* by using API 20 E or other phenotypic tests.

The three strains had identical *glnA* (492 bp), *gyrB* (502 bp), *recA* (512 bp), *HSP60* (523 bp) and 16S rRNA (1451 bp) gene sequences. In phylogenetic analyses of the housekeeping-gene sequences *glnA*, *gyrB*, *recA* and *HSP60*, they formed a unique group (Fig. 1; Supplementary Fig. S3, available in IJSEM Online) within the genus *Yersinia*. Depending on the analysis, either *Y. mollaretii* (Supplementary Fig. S3) or *Y.*

aldovae (Fig. 1) was located as the closest phylogenetic neighbour, with low (<40 %) bootstrap support. Phylogenetic analysis of the 16S rRNA gene sequences also grouped the strains together with *Y. aldovae* and *Y. mollaretii* (Fig. 2; Supplementary Fig. S4, available in IJSEM Online) with higher bootstrap support (>60 %), but the DNA–DNA hybridization results showed that the three strains belonged to neither *Y. aldovae* nor *Y. mollaretii*. Strains ÅYV7.1 KOH2^T and ÅYV25K1 had hybridization values of 32–47 and 29–39 % with *Y. aldovae* CIP 103162^T and *Y. mollaretii* DSM 18520^T, respectively. Strains Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T had 16S rRNA gene sequence similarity of 99.9 % to *Y. aldovae* CIP 103162^T. With the type strains of other *Yersinia* species, the 16 rRNA gene sequence similarity was 98.0–99.3 %.

Fig. 1. Neighbour-joining tree based on concatenated *glnA*, *gyrB*, *HSP60* and *recA* gene sequences of *Yersinia pekkanenii* sp. nov. and type and reference strains of the genus *Yersinia*. Bootstrap values (%) for 1000 replicates are given at branch points. *S. proteamaculans* was used as outgroup. Bar, 2 % difference in nucleotide sequence.

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences of *Yersinia pekkanenii* sp. nov. and type and reference strains of the genus *Yersinia*. Bootstrap values (%) for 1000 replicates are given at branch points. *S. proteamaculans* was used as outgroup. GenBank accession numbers are shown in parentheses. Bar, 1% difference in nucleotide sequence.

Numerical analyses of both *Hind*III and *Eco*RI ribopatterns supported the results of the housekeeping-gene analysis. Strains Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T formed a separate cluster within the genus *Yersinia* (Fig. 1). The DNA G + C contents of strains Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T were identical at 47.5 mol%.

It is proposed that strains Å125KOH2, ÅYV25K1 and ÅYV7.1KOH2^T represent a novel species in the genus *Yersinia*, for which the name *Yersinia pekkanenii* sp. nov. is suggested.

Description of *Yersinia pekkanenii* sp. nov.

Yersinia pekkanenii (pek.ka.ne'ni.i. N.L. gen. masc. n. *pekkanenii* in honour of the late Professor Timo Pekkanen, who was a veterinarian and former head of the Department of Food and Environmental Hygiene in the Faculty of Veterinary Medicine of the University of Helsinki. He made a substantial contribution to the development of scientific research of food hygiene in Finland).

Cells are Gram-negative rods of 1–3 µm diameter and are non-motile, oxidase-negative and catalase-positive. Positive for urea hydrolysis and ONPG utilization. Ferments D-xylose, cellobiose, trehalose, L-arabinose and glucose. Ferments lactose weakly. Does not ferment adonitol, sorbose, salicin, raffinose, glycerol, inositol, sorbitol, melibiose, rhamnose or sucrose. Negative for lysine decarboxylase, ornithine decarboxylase, Voges–Proskauer reaction, lipase and indole production. Does not hydrolyse aesculin. Grows on blood, tryptic soy, MacConkey and nutrient agar at 25–37 °C. Produces

pinpoint colonies on blood agar at 30 °C in 24 h. Colonies are circular and convex. DNA G + C content is 47.5 mol%. 16S rRNA gene sequence similarity with *Y. aldovae* CIP 103162^T is 99.9%.

The type strain is ÅYV7.1KOH2^T (=DSM 22769^T =LMG 25369^T), isolated from a lettuce sample. Reference strains are Å125KOH2 and ÅYV25K1, isolated from water and soil samples, respectively, suggesting an environmental habitat.

Acknowledgements

We are grateful to the Academy of Finland for supporting our research financially through the ELVIRA programme (decision no. 1117921) and through funding to the Finnish Centre of Excellence in Microbial Food Safety Research (decision no. 1118602). DVM Riitta Rahkila is acknowledged for her help during revision of the manuscript and we thank E. Merivirta for her technical assistance.

References

- Atlas, R. M. & Snyder, J. W. (2006). *Handbook of Media for Clinical Microbiology*, 2nd edn. Boca Raton, FL: CRC Press.
- Jakava-Viljanen, M., Murros, A., Palva, A. & Björkroth, K. J. (2008). *Lactobacillus sobrius* Konstantinov et al. 2006 is a later synonym of *Lactobacillus amylovorus* Nakamura 1981. *Int J Syst Evol Microbiol* **58**, 910–913.
- Kotetishvili, M., Kreger, A., Wauters, G., Morris, J. G., Jr, Sulakvelidze, A. & Stine, O. C. (2005). Multilocus sequence typing for studying genetic relationships among *Yersinia* species. *J Clin Microbiol* **43**, 2674–2684.

- Merhej, V., Adékambi, T., Pagnier, I., Raoult, D. & Drancourt, M. (2008).** *Yersinia massiliensis* sp. nov., isolated from fresh water. *Int J Syst Evol Microbiol* **58**, 779–784.
- Niskanen, T., Laukkanen, R., Murros, A., Björkroth, J., Skurnik, M., Korkeala, H. & Fredriksson-Ahomaa, M. (2009).** Characterisation of non-pathogenic *Yersinia pseudotuberculosis*-like strains isolated from food and environmental samples. *Int J Food Microbiol* **129**, 150–156.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989).** Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Sprague, L. D., Scholz, H. C., Amann, S., Busse, H.-J. & Neubauer, H. (2008).** *Yersinia similis* sp. nov. *Int J Syst Evol Microbiol* **58**, 952–958.
- Stackebrandt, E. & Liesack, W. (1993).** Nucleic acids and classification. In *Handbook of New Bacterial Systematics*, pp. 152–194. Edited by M. Goodfellow & A. G. O'Donnell. London, UK: Academic Press.
- Xu, H. X., Kawamura, Y., Li, N., Zhao, L., Li, T. M., Li, Z. Y., Shu, S. & Ezaki, T. (2000).** A rapid method for determining the G + C content of bacterial chromosomes by monitoring fluorescence intensity during DNA denaturation in a capillary tube. *Int J Syst Evol Microbiol* **50**, 1463–1469.