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Psychrobacillus vulpis sp. nov., a new species isolated from faeces of a red fox in Spain

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Abstract

A facultative anaerobic, chemoheterotrophic, endospore-forming, Gram-stain-positive rod, designated as strain Z8^T, was isolated from red fox (*Vulpes vulpes*) faeces sampled at Tablas de Daimiel National Park, Ciudad Real, Spain. Strain Z8^T grew at 0–37 °C (optimum, 28 °C), in the presence of 0–5.5% (w/v) NaCl (2.5%, w/v) and at pH 6–10 (pH 7). The strain was motile and positive for catalase, oxidase, H_2S and siderophore production, acid and alkaline phosphatases, and *N*-acetylglucosamine, adipic acid and malate assimilation. It hydrolysed starch, DNA, ʟ-tyrosine, Tween 20, Tween 80 and lecithovitellin. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain Z8^T is a member of the genus *Psychrobacillus*, showing high sequence similarity to *Psychrobacillus lasiicapitis* NEAU-3TGS17^T (99.2%) and *Psychrobacillus soli* NHI-2T^T (99.1%), and around 98% to other known species of the genus *Psychrobacillus*. Digital DNA–DNA hybridization and average nucleotide identity values were lower than 24 and 79%, respectively, with the most related species. *In silico* G+C content was 35.9 mol%. The major cellular fatty acids of strain Z8^T were iso-$C_{14:0}$, iso-$C_{15:0}$ and anteiso-$C_{15:0}$. The novel strain contained diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as predominant polar lipids, and the main respiratory isoprenoid quinone was MK-8. Based on the 16S rRNA phylogenetic analysis, together with MLSA (*recA, rpoB* and *gyrB*), phylogenomic, chemotaxonomic and phenotypic results, we demonstrate that strain Z8^T represents a novel species of the genus *Psychrobacillus,* for which the name *Psychrobacillus vulpis* sp. nov., is proposed. The type strain is Z8^T (=CECT 9721^T=LMG 31001^T).

The genus *Psychrobacillus* belongs to the family *Bacillaceae* within the phylum *Firmicutes*. The genus *Psychrobacillus* was proposed by Krishnamurthi [1] in 2010 after a re-examination of some species of the genus *Bacillus* by a polyphasic approach. Based on the rRNA gene sequence similarities of members of *Bacillus* rRNA group 2, *Bacillus insolitus* W16B^T, *Bacillus psychrotolerans* 3H1^T and *Bacillus psychrodurans* 68E3^T were transferred to the new genus *Psychrobacillus* and reclassified. Cells of the genus *Psychrobacillus* are Gram-positive, endospore-forming, motile rods and strictly aerobic. They have a cell-wall peptidoglycan of A4β type with ornithine as the diaminoacid at position 3 of the peptide subunit; MK-8 as the major menaquinone, followed by MK-7 and minor quantities of MK-6 and MK-9. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The major fatty acids are anteiso-$C_{15:0}$, iso-$C_{14:0}$ and $C_{16:1}\omega7c$ alcohol. The G+C content of the genomic DNA ranges from 35.7 to 36.6 mol% [1]. At the time of writing, the genus includes five validly described species: *Psychrobacillus insolitus* [1, 2], *Psychrobacillus psychrodurans* [1, 3], *Psychrobacillus psychrotolerans* [1, 3], *Psychrobacillus soli* [4] and *Psychrobacillus lasiicapitis* [5]. The type species is *P. insolitus* W16B^T [6].

During the course of a study focused on the isolation of chitin-degrading bacteria, a new strain, Z8^T, phylogenetically related to the genus *Psychrobacillus*, was isolated from the microbiota associated with red fox (*Vulpes vulpes*) faeces that were taken from Tablas de Daimiel National Park in Ciudad Real, Spain. Based on phenotypic, chemotaxonomic and phylogenetic data, together with a phylogenomic analysis, we propose in this paper to establish for this isolate the new species *Psychrobacillus vulpis* sp. nov.

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Keywords: *Psychrobacillus vulpis* sp. nov.; red fox; phylogenetic; chemotaxonomy; genome; polyphasic taxonomy.

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridrization; TSA, tryptic soy agar; TSB, tryptic soy broth.

The GenBank/EMBL/DDBJ accession numbers for the genome and 16S rRNA gene sequence of strain Z8^T are VDGI00000000 and MH910346, respectively.
Seven supplementary figures and one supplementary table are available with the online version of this article.

Strain Z8^T was isolated from a sample of faeces of a red fox (*Vulpes vulpes*) obtained in June 2015 from Tablas de Daimiel National Park, Ciudad Real, Spain (39° 08′ 17.1″ N 3° 41′ 49.8″ W). Faeces, which contained partially digested red swamp crayfish (*Procambarus clarkii*) debris, were place in a sterile recipient until laboratory processing. One gram of faeces was suspended in 0.9% (w/v) saline solution and heated at 80 °C for 20 min to select sporulated bacteria. Once the suspension had cooled down, serial dilutions were made (10^{-1} to 10^{-5}) and 100 µl of each dilution were plated on tryptic soy agar (TSA) and incubated at 28 °C for 7 days. The different isolates were subsequently plated and purified on the same medium. *P. insolitus* DSM 5^T, *P. psychrodurans* DSM 11713^T, *P. psychrotolerans* DSM 11706^T, *P. soli* NBRC 110600^T and *P. lasiicapitis* DSM 100484^T were used as reference strains for comparative analysis with strain Z8^T. All strains were maintained and cultivated in tryptic soy broth (TSB) medium at 28 °C, except *P. insolitus* DSM 5^T which was cultivated at 20 °C.

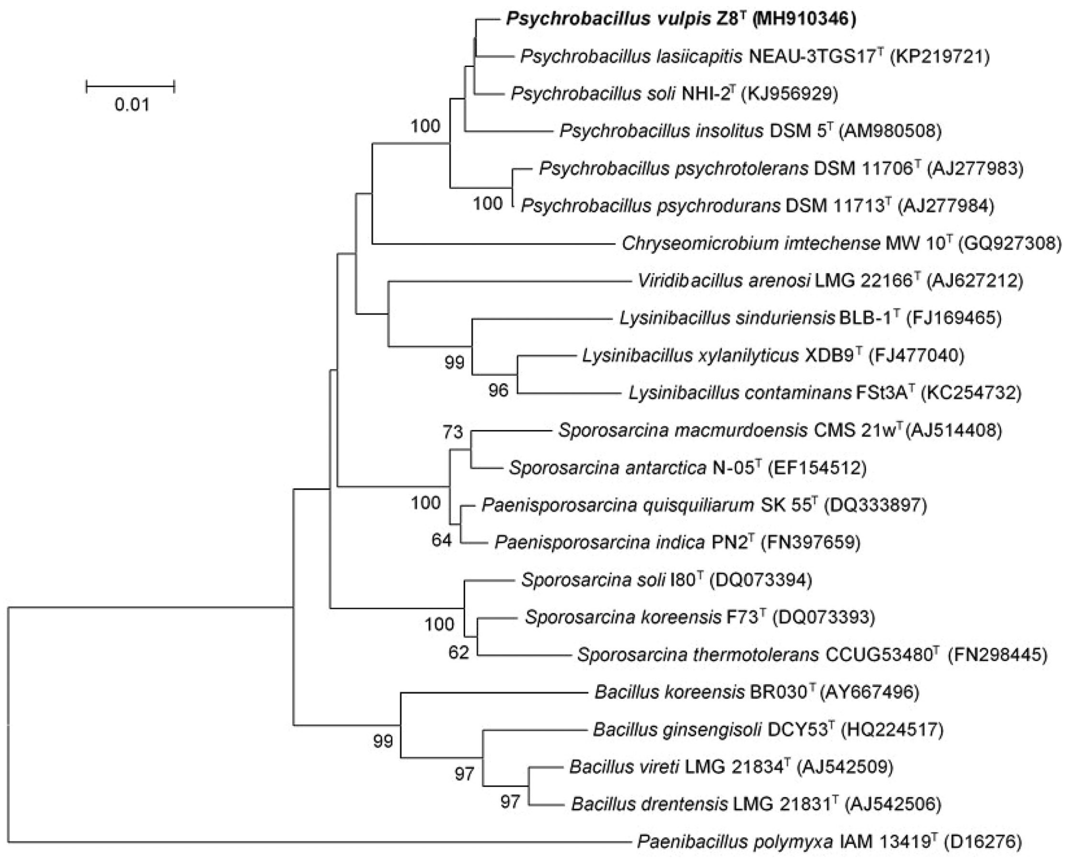
Genomic DNA was extracted from an overnight 5 ml culture of strain Z8^T in TSB medium using an X-DNA purification kit (Xtrem Biotech). The 16S rRNA gene was amplified by PCR using the universal bacterial primers 16F27 and 16R1488 [7]. The PCR product was purified and cloned into the pGEM-T vector (Promega). Direct sequencing of PCR-amplified DNA was determined using an ABI PRISM DyeTerminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) and an ABI PRISM 377 sequencer (Perkin-Elmer) according to the manufacturer's instructions. The almost full-length 16S rRNA sequence (1529 bp) obtained for strain Z8^T was compared to reference 16S rRNA gene sequences available in the GenBank and EMBL databases obtained from the NCBI genome database using BLASTN software [8] and the EzBioCloud server (www.ezbiocloud.net/) [9].

Phylogenetic trees based on 16S rRNA sequences were reconstructed by applying the neighbour-joining [10], maximum-parsimony [11] and maximum-likelihood [12] algorithms using Molecular Evolutionary Genetics Analysis (MEGA) software version X [13], after multiple data alignment by CLUSTAL Omega [14]. Bootstrap analysis (1000 replications) was applied to ensure cluster stability.

The 16 rRNA gene sequence analysis showed that strain Z8^T was a member of the genus *Psychrobacillus*. Its closest relatives were *P. lasiicapitis* DSM 100484^T and *P. soli* NBRC 110600^T with similarity values of 99.2 and 99.1%, respectively. The 16S rRNA sequence similarity to the rest of the members of genus *Paenibacillus* was near 98%. The 16S rRNA gene-based phylogenetic reconstruction by using the neighbour-joining method, including the sequences of the most related species obtained from the GenBank database, showed that Z8^T clustered in an independent branch with *P. lasiicapitis* DSM 100484^T (Fig. 1). Highly similar phylogenetic reconstructions were obtained using maximum-parsimony and maximum-likelihood algorithms (Figs S1 and S2, available in the online version of this article).

The draft genome sequences of strain Z8^T, *P. lasiicapitis* DSM 100484^T and *P. soli* NBRC 110600^T were determined in this study. Genomic DNA from the three strains was extracted according to the protocol described by Marmur [15] and sequenced using the Illumina Hi-Seq platform (2×150 bp paired-end reads) at the STAB VIDA facilities (Caparica, Portugal). The reads, which were processed by BBDuk (https://sourceforge.net/projects/bbmap/) to remove adapters and low-quality bases and reads, were then assembled using SPAdes software version 3.11.1 [16]. The strain Z8^T draft genome assembly lead to 4024205 bp in 76 contigs with a coverage of 120× and a G+C content of 35.9 mol%. In the case of the *P. lasiicapitis* DSM 100484^T draft genome, the size obtained was 4488252 bp in 28 contigs, with a coverage of 145× and a G+C content of 37.1 mol%. Finally, the draft genome of *P. soli* NBRC 110600^T showed a size of 4226526 bp into 101 contigs, with a coverage of 139× and a G+C content of 37.1%. All these genomes have been deposited in the NCBI genome database under de accession numbers: Z8^T (VDGI00000000), *P. lasiicapitis* DSM 100484^T (VDGH00000000) and *P. soli* NBRC 110600^T (VDGG00000000). The average nucleotide identity (ANI) values were determined between strain Z8^T and the other five genomes of the members of the genus *Psychrobacillus* (*P. lasiicapitis* DSM 100484^T, *P. soli* NBRC 110600^T, *P. psychrodurans* DSM 11713^T, *P. psychrotolerans* DSM 11706^T, *P. insolitus* DSM 5^T). The calculation was based on BLAST+ (ANIb) and Mummer from JSpecies (ANIm) [17]. OrthoANI was assessed with the aid of OrthoANI software [18]. The *in silico* DNA–DNA hybridization (DDH) values between strain Z8^T and the other five *Psychrobacillus* species were estimated using the recommended BLAST+ algorithm in the Genome-to-Genome Distance Calculator (GGDC 2.1 software) of DSMZ [19] . The ANI values based on ANIb, ANIm, orthoANI and DDH between strain Z8^T and the other members of *Psychrobacillus* genus are shown in Table S1. ANIb values between strain Z8^T and *P. lasiicapitis* DSM 100484^T and *P. soli* NBRC 110600^T were 79.0 and 79.1%, respectively. In the case of ANIm, the results were 84.6 and 84.3%, respectively. The rest of the ANIb and ANIm results of strain Z8^T against the other species of the genus as well as OrthoANI values were lower than 80.0%. The accepted threshold for species delimitation using ANIb, ANIm, orthoANI is 95–96% [20]. The *in silico* DDH results were in all cases lower than 24%, a value lower than the cut-off value for species delineation (70%) [21]. Both ANI and DDH results denote that strain Z8^T is a new species of the genus *Psychrobacillus*.

In order to perform a more robust analysis than that obtained using the 16S rRNA gene sequence, a multilocus sequence analysis (MLSA) was done using the concatenated sequence of the genes 16S rRNA, *recA*, *rpoB* and *gyrB*. In the case of *P. psychrodurans* DSM 11713^T, *P. psychrotolerans* DSM 11706^T and *P. insolitus* DSM 5^T, for which genome sequences were previously published, these gene sequences were obtained from their NCBI deposited genomes with accession numbers NZ_FOUN00000000.1, NZ_FOXU00000000 and NZ_QKZI00000000, respectively. With the aim of extracting these gene sequences from the strain Z8^T, *P. lasiicapitis* and *P. soli* genomes, annotation with Rapid Annotation using Subsystem Technology (RAST) [22] was done. However, as the 16S rRNA



AQ2

Fig. 1. Phylogenetic reconstruction of strain Z8^T and its closest relatives based on 16S rRNA sequences by using the neighbour-joining algorithm. GenBank/EMBL/DDBJ accesion number of each sequence is shown in parenthesis. Bootstrap values are expressed as percentages of 1000 replications, and those over 60% are shown at branch points. Bar, 0.01 substitutions per nucleotide position. Asterisks indicate that the corresponding nodes were also obtained in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. The *Paenibacillus polymyxa* IAM 13419^T 16S rRNA sequence was used as an outgroup.

gene sequences of *P. lasiicapitis* and *P. soli* were not complete, the 16S rRNA gene sequences of these species deposited in the NCBI were used. The phylogenetic tree reconstruction was performed by using the neighbour-joining method in MEGA X [13] with *Paenibacillus polymyxa* IAM 13419^T as an outgroup. Concatenation of the 16S rRNA gene and the three housekeeping genes (*recA*, *rpoB* and *gyrB*) of the six *Psychrobacillus* species and the outgroup resulted in an alignment of 8106 nucleotides. The reconstructed phylogenetic tree from MLSA (Fig. S3) showed the same distribution as that from the 16S rRNA gene reconstruction, demonstrating that strain Z8^T belongs to the genus *Psychrobacillus* and that this strain forms an independent cluster from *P. lasiicapitis* DSM 100484^T and *P. soli* NBRC 110600^T with a higher robustness than the one obtained with 16S rRNA gene sequences.

A core and pan genome reconstruction of the members of the genus *Psychrobacillus* was carried out using the Bacterial Pan Genome Analysis [23] tool, with default settings and phylogeny based on multi-locus sequence typing of the core genes. Once the core genome of the genus was obtained, a phylogenetic tree was performed with MEGA X using the neighbour-joining method. The results agreed with those obtained previously (Fig. 2). Analysis of the core genome resulted in a total of 1773 core orthologous genes in a total pangenome of 8225 genes.

Finally, the genomes of all *Psychrobacillus* species were compared using BLAST Ring Image Generator software [24] (Fig. S4), showing the differences between the strains, with zones present in strain Z8^T that were not found in the other species of the genus.

The phenotypic characterization of strain Z8^T was carried out following the recommended minimal standards for describing new taxa of aerobic, endospore-forming bacteria [25]. Colony morphology, size and pigmentation were analysed after 48 h incubation at 28 °C on TSA. To study morphology, cell size and the presence of flagella, strain Z8^T was grown in TSB medium at 28 °C on a shaking incubator at 100 r.p.m. and examined by optic microscopy and TEM. Sporulation was also investigated from a 10 days culture in Schaeffer sporulation medium [26] at 28 °C by optic microscopy after Schaeffer–Fulton staining [27]. Motility was evaluated by the hanging-drop method [28]

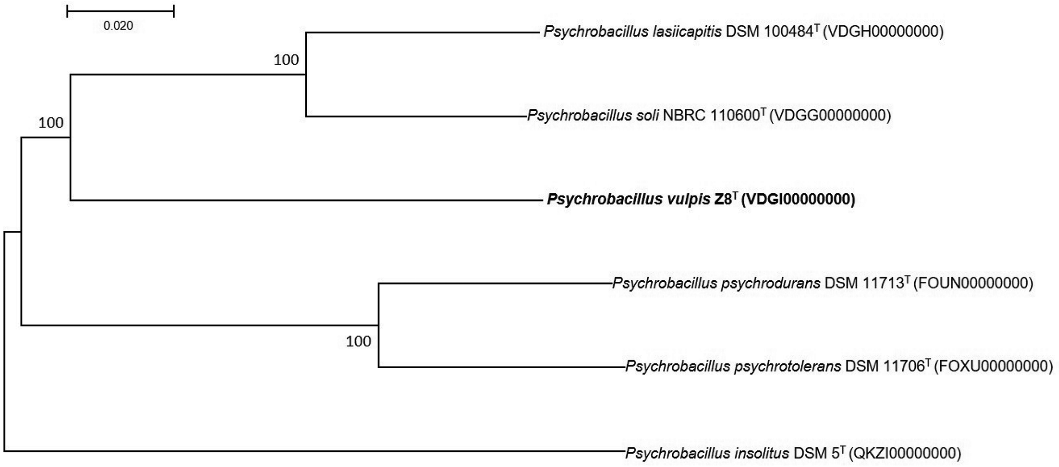


Fig. 2. Phylogenetic tree based on core orthologous proteins of the genus *Psychrobacillus* members. Phylogeny was inferred by using the neighbour-joining method and bootstrap values are expressed as percentages of 1000 replications near the branches and those over 60% are shown. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter=1) Bar, 0.02 substitutions per nucleotide position.

AQ3

using a log phase culture. Optimum growth conditions of salinity were determined in TSB medium with different NaCl concentrations (0.5, 1, 3, 5, 7.5 and 10% w/v). The optimal (and range) growth pH of strain Z8^T was evaluated by growing the strain under different pH values (pH 4, 5, 6, 7, 8, 9, 10 and 11). These pH values were reached using the following buffer systems: 0.1 M citric acid/0.1 M sodium citrate (pH 4.0–5.0,); 0.1 M KH_2PO_4/0.1 M NaOH (pH 6.0–8.0); 0.1 M $NaHCO_3$/0.1 M Na_2CO_3 (pH 9.0–10.0); 0.2 M KH_2PO_4/0.1 M NaOH (pH 11.0) [29]. The effect of different temperatures (0, 15, 22, 28, 32, 37 and 45 °C) on growth was evaluated on TSA. All biochemical tests were performed in TSB medium, pH 7 at 28 °C, unless otherwise stated. Indole production was tested using peptone broth as growth medium, while the Voges–Proskauer test used peptone broth supplemented with 0.5% (w/v) glucose [30, 31]. Hydrolysis of aesculin, cellulose, colloidal chitin, starch, ʟ-tyrosine, Tween 20, Tween 80 and urea, production of acid and alkaline phosphatase, caseinase, catalase, DNAase, haemolysine, lecithinase, liquefaction of gelatine, oxidase and production of H_2S and siderophores were evaluated as described elsewhere [32–42]. Growth under anaerobic conditions was determined on TSA medium by incubation in hermetic jars using the Gas Pak Anaerobic System (BBL) to generate an anaerobic atmosphere over 2 week period. Other phenotypic characteristics were analysed by using API 20NE, API ZYM and API 50CH strip systems, following the manufacturer's instructions. Antimicrobial susceptibility was evaluated by the diffusion agar method [43]. The following antimicrobial discs were tested (µg ml^{-1}): ampicillin (10), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30), novobiocin (30), penicillin G (10), rifampicin (2), tetracycline (30) and sulfamethoxazole/trimethoprim (23.75/1.25).

The strain Z8^T colony morphology is vortex-like and transparent-grey coloured (Fig. S5). Cells of strain Z8^T were Gram-stain-positive, endospore-forming and rod-shaped with a size range of 1.8–2.1×0.6–0.7 µm. Endospores in swollen sporangia were central and rounded with a size range of 0.8–0.9×0.8–0.9 µm (Fig. S6). Cells were motile by flagella. It grew from 0 to 5.5% (w/v) NaCl, with an optimum at 2.5%; the growth pH range was from pH 6 to 10, with an optimum at pH 7. The temperature range for growth was 0–37 °C, while the optimum temperature was 28 °C. It was also able to grow under anaerobic conditions. Strain Z8^T gave positive results for oxidase, catalase, H_2S and siderophore production, and alkaline and acid phosphatases. It was able to hydrolyse starch, Tween 20, Tween 80, lecithovitellin, DNA and ʟ-tyrosine. By contrast, strain Z8^T was negative for indole and Voges–Proskauer tests and for cellulose, casein, gelatin, chitin, haem, urea and aesculin hydrolysis. In the case of API tests, strain Z8^T was positive for nitrate reduction and for assimilation of N-acetylglucosamine, adipic acid, malate, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, alpha-chymotripsin and naphthol-AS-BI-phosphohydrolase. A weakly positive result was obtained for valine arylamydase. No acid production was detected for any sugar in the API 50CH kit. In the case of the antimicrobial susceptibility test, strain Z8^T was susceptible to all the compounds assayed except for nalidixic acid, for which it was resistant, and kanamycin, for which it was intermediately susceptible. The main differences among strain Z8^T and the closest related strains are represented in Table 1. Other characteristics of strain Z8^T are given in the species description.

Polar lipids, respiratory quinones and cellular fatty acids were analysed from a 24 h culture in TSB medium of strain Z8^T at 28 °C. In the case of *P. lasiicapitis* DSM 100484^T and *P. soli*

Table 1. Differential characteristics between strain Z8^T and other members of the genus *Psychrobacillus*

Strains: 1, 1Z8^T; 2, *Psychrobacillus lasiicapitis* DSM 100484^T; 3, *Psychrobacillus soli* NBRC 110600^T; 4, *Psychrobacillus psychrodurans* DSM 11713^T; 5, *Psychrobacillus psychrotolerans* DSM 11706^T; 6, *Psychrobacillus insolitus* DSM 5^T. All data were obtained from this study. +, Positive; −, negative; w, weakly positive. All strains were motile and postitive for lechitovitellin hydrolysis, acid phosphatase, siderophore production, esterase (c4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin and naphthol-AS-BI-phosphohydrolase. All strains were negative for indole and Voges–Prokauer tests, casein, cellulose, chitin and haem hydrolysis, arginine dihydrolase, assimilation of glucose, arabinose, mannose, mannitol, maltose, potassium gluconate, capric acid, citrate and phenylacetic acid. All strains tested were negative for lipase (C14), α-galactosidase, *β*-galactosidase, *N*-acetyl-ß-glucosaminidase, α-mannosidase and α-fucosidase. None of the strains produced acids from glycerol, erythrol, D-arabinose, L-arabinose, L-xylose, adonitol, methyl *β*-D-xyloside, galactose, manose, sorbose, rhamnose, dulcitol, inositol, sorbitol, 1-methyl-D-mannoside, 1-methyl-D-glucoside, arbutin, salicin, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, glycogen, xylitol, gentibiose, turanose, D-xylose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate and 2-keto-gluconate.

Characteristic	1	2	3	4	5	6
Colony morphology	Vortex-like	Round	Round	Round	Round	Round
NaCl range for growth (%; w/v)	0–5.5	0–6.5	0–6.5	0–6.5	0–3.5	0–3.5
NaCl optimum for growth (%; w/v)	2.5	1.5	1.5	1.5	2.5	1.5
pH range for growth	6–10	7–10	6–10	7–10	8–10	7–9
pH optimum for growth	7	7	6	8	8	7
Temperature range for growth (°C)	0–37	0–35	0–40	0–30	0–30	0–25
Temperature optimum for growth (°C)	28	28	28	28	28	20
Hydrolysis of:						
Gelatin	−	−	+	+	−	−
DNA	+	+	−	+	+	−
L-Tyrosine	+	−	−	−	−	−
Urea	−	+	+	−	−	−
Asimilation of:						
N-Acetyl-glucosamine	+	−	−	+	−	−
Adipic acid	+	−	+	−	−	−
Malate	+	+	+	−	−	−
Enzymes (API ZYM):						
Valine arylamidase	w	−	+	−	+	+
Cystine arylamidase	−	−	+	−	+	−
β-Glucuronidase	−	+	−	−	−	−
Acid production from:						
D-Glucose	−	−	w	+	−	−
Starch	−	−	+	+	−	−

NBRC 110600^T, data of polar lipids, respiratory quinones and cellular fatty acids were obtained from the original descriptions for each species [4, 5]. Analysis of polar lipids and respiratory quinones were conducted by the Identification Service of the DSMZ, Braunschweig, Germany, according to the protocol previously described by Bligh and Dyer [44] and Tindall [45, 46], respectively. The fatty acid analyses were carried out at the Spanish Type Culture Collection (CECT) in Valencia, Spain. The whole-cell composition of fatty acids was determined by using an Agilent 6850 gas chromatograph [47] and then analysed with the aid of the MIDI Microbial Identification System and the TSBA6 database.

The analysis of the polar lipids of strain Z8^T showed that the main component was diphosphatidilglycerol followed by phosphatidylglycerol and phosphatidylethanolamine (Fig. S7), as has been described in other *Psychrobacillus* species [5]. The major fatty acids were anteiso-C$_{15:0}$ (49.8%), iso-C$_{15:0}$ (22.7%), iso-C$_{14:0}$ (12.3%) and a low amount of C$_{16:1}$ω7*c* alcohol (2.2%), a fatty acid profile similar to that of other members of the genus *Psychrobacillus*. The menaquinones found were MK-8 (81%), MK-7 (18%) and MK-6 (traces).

DESCRIPTION OF *PSYCHROBACILLUS VULPIS* SP. NOV.

Psychrobacillus vulpis (vul′pis. L. gen. n. *vulpis*, of a fox).

Cells are rod, Gram-stain-positive, motile, facultative anaerobic and able to sporulate. Endospores in swollen sporangia are central and rounded with a size range of 0.8–0.9×0.8–0.9 µm. Colonies are transparent-grey, vortex-like shape and invade all of the plate after 5 days of incubation at 28 °C. Slightly halophilic bacteria with a NaCl range from 0 to 5.5% (w/v) and an optimum at 2.5% (w/v). Able to grow in a pH range of pH 6–10 and from 0 to 37 °C, with optima at pH 7 and 28 °C. Positive for catalase, oxidase and H_2S production and negative for Voges–Proskauer and indole production. Starch, Tween 20, Tween 80, DNA and L-tyrosine are hydrolysed, but not cellulose, casein, gelatine, chitin, haem and aesculin. Positive for both alkaline and acid phosphatases, and siderophores production. Nitrate reduction is positive but negative for arginine dehydrolase, β-galactosidase (PNPG) and urease. No assimilation was detected for any API 20NE compound except for *N*-acetylglucosamine, malate and adipic acid. Positive for esterase (C4) and for esterase lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin and naphthol-AS-BI-phosphohydrolase. Weakly positive for valine arylamidase. No acid production from any substrate in the API 50CH assay kit. The major cellular fatty acids are iso-$C_{14:0}$, iso-$C_{15:0}$ and anteiso-$C_{15:0}$. The main polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol, and the main respiratory isoprenoid quinone is MK-8 with a lower amount of MK-7. The DNA G+C content of the type strain is 35.9 mol%.

The type strain is Z8^T (=CECT 9721^T=LMG 31001^T), isolated from red fox (*Vulpes vulpes*) faeces collected in Las Tablas de Daimiel National Park, Ciudad Real, Spain.

The GenBank/EMBL/DDBJ accession number for 16S rRNA sequence of *Psychrobacillus vulpis* Z8^T is MH910346, and the complete genome is deposited under the accession number VDGI00000000.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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