

Description of *Stenotrophomonas sepilia* sp. nov., isolated from blood culture of a hospitalized patient as a new member of *Stenotrophomonas maltophilia* complex

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Abstract

Stenotrophomonas sepilia strain SM16975 (= JCM 32102; = KCTC 62052) is a new species isolated from the blood culture of a hospitalized patient. The biochemical characterization, phenotypic criteria, phylogenomic reconstruction, and genomic analysis were carried out to differentiate it from its phylogenetic neighbours, establishing novel species status in the genus *Stenotrophomonas* and within *Stenotrophomonas maltophilia* complex (Smc).

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Introduction

The genus *Stenotrophomonas* was described with *S. maltophilia* as type species, which was earlier isolated from the pleural fluid and named as *Bacterium bookeri*, which was further classified as *Pseudomonas maltophilia* [1,2]. Further, it was transferred to the genus *Xanthomonas* [3] and finally to the new genus *Stenotrophomonas* [1]. At the time of writing, the genus *Stenotrophomonas* comprises 18 species with validly published names isolated from a large range of natural and artificial environments and geographical regions (<https://lpsn.dsmz.de/genus/stenotrophomonas>). These 18 species include *S. maltophilia*, *S. africana*, *S. nitritireducens* [4], *S. acidaminiphila* [5], *S. rhizophila*

[6], *S. koreensis* [7], *S. humi*, *S. terrae* [8], *S. chelatiphaga* [9], *S. ginsengisoli* [10], *S. panacihumi* [11], *S. daejeonensis* [12], *S. pavanii* [13], *S. tumulicola* [14], *S. bentonitica* [15], *S. pictorum* [16], *S. lactitubi* and *S. indicatrix* [17].

The genus *Stenotrophomonas* is one of the rapidly expanding genera having biotechnological importance with only the exception of *S. maltophilia*, which has globally emerged as a multi-drug resistant opportunistic pathogen [18,19]. Due to 16S rRNA gene sequence conservation, taxonomy of *S. maltophilia* is complicated. Apart from the validly described species, there are misclassified species also that are associated with the genus *Stenotrophomonas* and include *Pseudomonas hibiscicola*, *P. beteli*, and *P. geniculata*, which are considered as synonyms of *S. maltophilia* [20]. Along with these misclassified species, *S. maltophilia*, *S. africana*, and *S. pavanii* belong to the *S. maltophilia* complex (Smc), which is a group of closely related species that cannot be resolved by 16S rRNA gene-based phylogeny. Although rep-PCR, *gyrB* and multi-locus sequence typing have revealed high diversity in strains of *S. maltophilia* [20–23], they lack in resolving true phylogeny of *Stenotrophomonas*.

The advent of the genomic era has radically transformed our understanding of bacterial taxonomy and evolution. Taxonogenomics and phylogenomics provide us intra-species

and strain level resolution. Genome-derived criteria such as average nucleotide identity (ANI), average amino acid identity (AAI), and digital DNA-DNA hybridization (dDDH) with species-level cut-offs of 95%, 95%, and 70%, respectively [24–27]. Genome resources of the family *Lysobacteraceae* can help better to resolve and demarcate species of the genus *Stenotrophomonas* [28,29]. As a part of our previous study, we aimed to study the strain level diversity of clinical isolates of *S. maltophilia* from hospitalized patients by whole-genome sequencing and taxonogenomic studies using reference strains of the genus *Stenotrophomonas* [30]. On the basis of species delineation whole-genome similarity parameters that included ANI and dDDH cut-offs, we found five cryptic novel genomospecies (genomospecies 2–6) clustering along with *S. maltophilia* (genomospecies 1) that further constitutes the *S. maltophilia* complex (Smc). The strain SM16975^T was part of that study, and based on the phylogenomics, ANI, and dDDH, it is considered as the putative novel genomospecies of the Smc along with the eight other isolates, which were designated under genomospecies 3. Genomospecies 3 is the second most dominant after genomospecies 1, which includes *S. maltophilia* among the total isolates under study. We have also generated the whole-genome resource of type strains of the genus *Stenotrophomonas* [31], which can be used for genome-based classification. Herein, we are describing genomospecies 3 as novel species of the genus *Stenotrophomonas*.

Isolation and growth conditions

The strain SM16975^T was isolated from the blood specimen of a 28-year-old male patient admitted in cardiothoracic vascular surgery intensive care unit of a tertiary care hospital, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, Northern part of India (30.7650° N, 76.7750° E), in September 2012. The strain SM16975^T was recovered from a blood specimen by using a continuous monitoring blood culture system BACTEC 9240 (BD Diagnostics, USA). Out of 46,243 blood cultures performed from April 2012 to March 2013, a total of 33 isolates of *S. maltophilia* were obtained [32].

Phenotypic and biochemical characteristics

Colonies were large, smooth, convex, glistening, circular with lavender-green pigment on blood agar. Colourless colonies were obtained on MacConkey agar as it is a non-lactose fermenting organism [33].

Bacterial cells were Gram-negative, rod-shaped, motile, catalase-positive, and oxidase-negative. Antibiotic susceptibility profile was determined on Mueller–Hinton agar (Oxoid) by Kirby Bauer disk diffusion method according to the breakpoints of the Clinical and Laboratory Standards Institute (CLSI) (<http://www.clsi.org>) approved standards. [CLSI. 2020. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI M100. Clinical and Laboratory Standards Institute, Wayne, PA.] The strain SM16975^T was sensitive to co-trimoxazole, levofloxacin, minocycline, and resistant to ceftazidime by MIC.

Bacteria display extensive diversity in fatty acid chains and fatty acids are major chemotaxonomic markers. The fatty acid analysis is particularly useful in the case of closely related species with uniform phenotypic characteristics [34]. As SM16975^T is closely related to *S. maltophilia* and was originally misclassified as *S. maltophilia*, we use fatty acid analysis to inspect the novel position of SM16975^T. For fatty acid analysis, strains were grown on tryptic soy broth agar (TSBA) medium for 24 hours at 37 °C. Total fatty acids of cells were separated from a loopful of culture as methyl esters using the method as described (Buyer 2002). Like Smc type strains, the fatty acid profile of SM16975^T has predominated with unsaturated fatty acids iso-C15:0 and anteiso-C15 (Table 1). Further, iso-C17:0, iso-C13:0 3-OH, summed features 3 and 8 were present in significant amounts in SM16975^T as compared to other strains of Smc. However, C16:1 w9c was found in low quantity in SM16975^T as compared to other Smc strains.

Biochemical characterization such as carbohydrate utilization, acid production, and various enzymatic activities was performed using BIOLOG GEN III MICROPLATETM on OMNILOG GEN III system (BIOLOG) according to manufacturer's instructions (Table 2). Strains belonging to Smc along with SM16975^T were investigated. Smc strains are able to utilize Dextrin, D-maltose, D-cellobiose, gentiobiose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, α-D-glucose, D-mannose, D-fructose, 1% sodium lactate, D-serine, D-fructose-6-phosphate, gelatin, glycyl-L - proline, L-lactic acid, citric acid, L-malic acid, bromo-succinic acid, propionic acid, acetic acid. Strains were resistant to antibiotics like rifamycin SV, troleandomycin, lincomycin, vancomycin, aztreonam.

Strain identification and phylogenomics

In an earlier study, we reported the genome of strain SM16975^T with NCBI Accession number LXXZ00000000 [30]. The genome is 4,582,512 bp long with a 66.4 % G + C content. The complete 16S rRNA gene was extracted from the genome by using RNAmmer [35], and the phylogenetic tree was constructed with all the other validly defined species along with

TABLE 1. Cellular fatty acid profile of strain SM16975^T and its comparison to the other type strains of related species in the genus *Stenotrophomonas*

Fatty acid	1	2	3	4	5	6
Straight chain fatty acid						
C10:0	0.68	1.49	0.89	0.87	0.94	1.81
C14:0	2.43	3.58	4.57	2.69	3.05	4.51
C16:0	10.83	12.14	12.88	7.29	11.96	12.35
C18:0	0.32	2.12	0.61	0.59	0.63	0.33
Branched fatty acid						
Iso C11:0	3.45	3.04	2.56	2.64	2.77	3.35
Iso C13:0	0.36	0.21	0.34	0.2	0.35	0.41
Iso C14:0	0.7	0.35	0.88	0.46	0.59	0.42
Anteiso C11:0	0.12	0.13	0.1	0.14	0.20	0.21
Anteiso C13:0	0.22	-	0.14	-	0.13	0.3
Iso C15:0	36.92	23.47	26.19	26.85	26.48	21.98
Iso C15:1 F	0.63	0.82	0.91	0.62	0.67	0.64
Anteiso-C15:0	15.42	10.16	11.62	16.60	15.67	14.45
Iso C16:0	1.69	0.68	1.5	1.07	1.46	0.82
Iso C17:0	4.27	2.91	2.38	3.53	3.71	2.30
Anteiso-C17:0	0.54	0.39	0.3	0.77	0.76	0.53
Iso C19:0	0.27	0.32	0.10	0.3	0.35	-
Unsaturated fatty acid						
C14:1 w5c	0.1	0.22	0.13	0.17	0.12	0.26
C16:1 W9c	1.49	5.66	5.15	4.59	4.03	6.59
C17:1w8c	0.13	-	0.24	0.24	0.29	-
C18:1w9c	1.06	3.21	1.88	2.37	2.77	1.91
C18:3 W6c	-	-	-	0.72	-	-
Hydroxy fatty acid						
C10:0 3-OH	0.13	0.19	0.24	-	0.06	0.06
C12:0 3-OH	2.32	3.05	3.38	1.46	1.71	1.06
C13:0 2-OH	0.51	0.24	0.4	0.3	0.39	-
Iso C11:0 3-OH	1.75	1.14	1.32	0.75	0.78	0.66
iso-C13:0 3-OH	2.75	1.69	1.94	1.01	1.88	0.38
Summed feature						
3	6.18	17.68	14.04	17.42	11.79	18.32
8	0.56	1.57	1.01	1.7	1.42	0.98
9	3.47	3.55	3.83	4.03	3.98	2.97

1. SM16975^T; 2. *S. maltophilia* ATCC 13637^T; 3. *P. geniculata* JCM 13324^T; 4. *Pseudomonas hibiscicola* JCM 13361^T; 5. *S. pavanii* DSM 25135^T; 6. *P. beteli* LMG 00978^T. Summed feature 3 comprises 16:1 w7c/16:1 w6c, summed feature 8 comprises 18:1 w7c, and summed feature 9 comprises 17:1 iso w9c.

misclassified species of the genus *Stenotrophomonas*. Further, 16S rRNA of SM16975^T had 99.73%, 99.58%, and 99.52% identities with *S. pavanii*, *P. beteli*, and *S. maltophilia*, respectively. The 16S rRNA-based identity and phylogenetic analysis suggested that the strain SM16975^T belongs to the Smc of genus *Stenotrophomonas* (Fig. 1).

For more detailed phylogenetic analysis, we constructed core genome-based phylogeny using the PhyML v3.0 [36], which uses the core genome fetched using the Mauve v20150226 [37] to perform phylogenetic construction. The Phylogenetic tree obtained using PhyML [36] with the type strains of the genus *Stenotrophomonas* placed the strain SM16975^T into a separate clade Fig. 2.

Taxonomgenomics

The degree of genome similarity of strain SM16975^T with the closely related species of genus *Stenotrophomonas* was estimated using average nucleotide identity (ANI) [38] using Jspecies [24], orthoANI using OAU [39], Average amino acid

TABLE 2. Comparison of biochemical characteristics of strain SM16975^T and the type strains of related species in the genus *Stenotrophomonas*

	1	2	3	4	5	6
Dextrin	+	+	+	+	+	+
D-Maltose	+	+	+	+	+	+
D-Trehalose	-	+	+	-	+	+
D-Cellobiose	+	+	+	+	+	+
Gentiobiose	+	+	+	+	+	+
Sucrose	+	+	+	-	+	+
D-Turanose	-	-	+	-	+	+
Stachyose	-	-	-	-	-	-
pH 6	+	+	+	+	+	+
pH 5	+	-	-	-	-	-
D-Raffinose	-	-	-	-	-	-
α-D-Lactose	-	-	+	-	-	+
D-Melibiose	+	+	+	+	+	+
β-Methyl-D-Glucoside	+	-	+	-	+	+
D-Salicin	+	-	+	+	+	+
N-Acetyl-D-Glucosamine	+	+	+	+	+	+
N-Acetyl-β-D-Mannosamine	-	+	-	+	+	-
N-Acetyl-D-Galactosamine	+	+	+	+	+	+
N-Acetyl Neuraminic Acid	-	-	-	-	-	-
1% NaCl	+	+	+	+	+	+
4% NaCl	+	+	+	+	+	+
α-D-Glucose	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+
D-Galactose	-	+	-	+	-	-
3-Methyl Glucose	-	-	-	-	-	-
D-Fucose	+	+	-	+	+	-
L-Fucose	-	-	-	-	-	-
L-Rhamnose	-	-	-	+	-	-
Inosine	-	-	-	-	-	-
1% Sodium Lactate	+	+	+	+	+	+
Fusidic Acid	-	+	+	+	+	+
D-Serine	+	+	+	+	+	+
D-Sorbitol	-	-	-	-	-	-
D-Mannitol	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-	-
myo-Inositol	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-
D-Glucose6-PO4	-	+	+	+	+	+
D-Fructose6-PO4	+	+	+	+	+	+
D-Aspartic Acid	-	-	-	-	-	-
D-Serine	-	+	-	+	-	-
Troleandomycin	+	+	+	+	+	+
Rifamycin SV	+	+	+	+	+	+
Minocycline	-	-	-	-	-	-
Gelatin	+	+	+	+	+	+
Glycyl-L-Proline	+	+	+	+	+	+
L-Alanine	+	+	+	+	+	+
L-Arginine	-	+	-	+	-	-
L-Aspartic Acid	-	+	-	+	-	-
L-Glutamic Acid	-	+	-	+	-	-
L-Histidine	-	-	-	-	+	-
L-Pyroglytamic acid	-	-	-	-	-	-
L-Serine	+	+	+	+	+	+
Lincomycin	+	+	+	+	+	+
Guanidine HCl	+	+	+	+	+	+
Niaproof 4	+	+	+	+	+	+
Pectin	-	-	-	+	+	+
D-Galacturonic acid	+	+	+	+	+	+
L-Galactonic Acid Lactone	-	-	-	+	-	-
D-Gluconic Acid	-	-	-	-	-	-
D-Glucuronic acid	+	+	+	-	+	+
Glucuronamide	+	+	+	+	+	+
Mucic Acid	-	+	-	+	-	-
Quinic Acid	-	-	-	+	-	-
D-saccharic Acid	-	-	-	+	-	-
Vancomycin	+	+	+	+	+	+
Tetrazolium Violet	+	+	+	+	+	+
Tetrazolium blue	+	+	+	+	+	+
p-hydroxy phenylacetic acid	-	-	-	-	-	-
Methyl Pyruvate	+	+	+	-	-	+
D-Lactic Acid Methyl Ester	-	+	-	+	-	-
L-lactic Acid	+	+	+	+	+	+
Citric Acid	+	+	+	+	+	+
α-Keto-Glutaric acid	+	+	+	+	+	+
D-Malic Acid	-	+	-	+	-	-
L-Malic Acid	+	+	+	+	+	+
Bromo-Succinic acid	+	+	+	+	+	+
Nalidixic Acid	-	+	+	+	+	+
Lithium Chloride	+	+	+	+	+	+
Potassium tellurite	-	-	-	-	+	-
Tween 40	+	+	+	+	+	+

Continued

TABLE 2. Continued

	1	2	3	4	5	6
γ-Amino-Butyric acid	-	-	-	-	-	-
α-Hydroxybutyric Acid	+	+	-	+	-	-
β-Hydroxy-D, LButyric Acid	-	-	-	+	-	-
α-Keto-Butyric acid	+	+	-	+	-	-
Acetoacetic Acid	+	+	-	+	-	-
Propionic Acid	+	+	+	+	+	+
Acetic Acid	+	+	+	+	+	+
Formic Acid	-	-	-	+	-	-
Aztreonam	+	+	+	+	+	+
Sodium Butyrate	+	+	+	+	+	+
Sodium Bromate	-	-	-	-	-	-

1. SM16975^T, 2. *Pseudomonas hibiscicola* JCM 13361^T, 3. *P. geniculata* JCM 13324^T, 4. *S. maltophilia* ATCC 13637^T, 5. *P. beteli* LMG 00978^T, 6. *S. pavanii* DSM 25135^T Symbols represents; '+': positive, '-': negative.

identity (AAI) using CompareM (<https://github.com/dparks1134/CompareM>) and digital DNA-DNA Hybridization [40] using GGDC online server (https://ggdc-test.dsmz.de/ggdc_background.php). The similarity values of ANI, orthoANI, AAI, and dDDH were 91.98%, 92.15%, 94.49%, and

46.4%, respectively (Table 3). It is pertinent to mention that values for all the taxonogenomic parameters were below the recommended cut-off values, depicting the novelty of the strain *S. sepilia* SM16975^T.

Stenotrophomonas sepilia (*sepilia* referring to sepsis in the given patient known to be caused by this organism and isolated from blood specimen). Based on differential biochemical tests, fatty acid composition, along with genome-based taxonomic criteria, we propose to classify this strain as a member of a new species within the genus *Stenotrophomonas*, family *Lysobacteraceae*, phylum *Proteobacteria*.

Furthermore, *S. sepilia* is the third clinical species apart from *S. maltophilia* and *S. africana* to be formally reported from the genus. As revealed in earlier studies [30,31,41], it is pertinent to formally start referring *S. maltophilia* and closely related *S. geniculata*, *S. africana*, *S. maltophilia*, *P. hibiscicola*, *S. sepilia*, *S. pavanii* that form a monophyletic clade based on core genome phylogeny as *Stenotrophomonas maltophilia* complex (Smc).

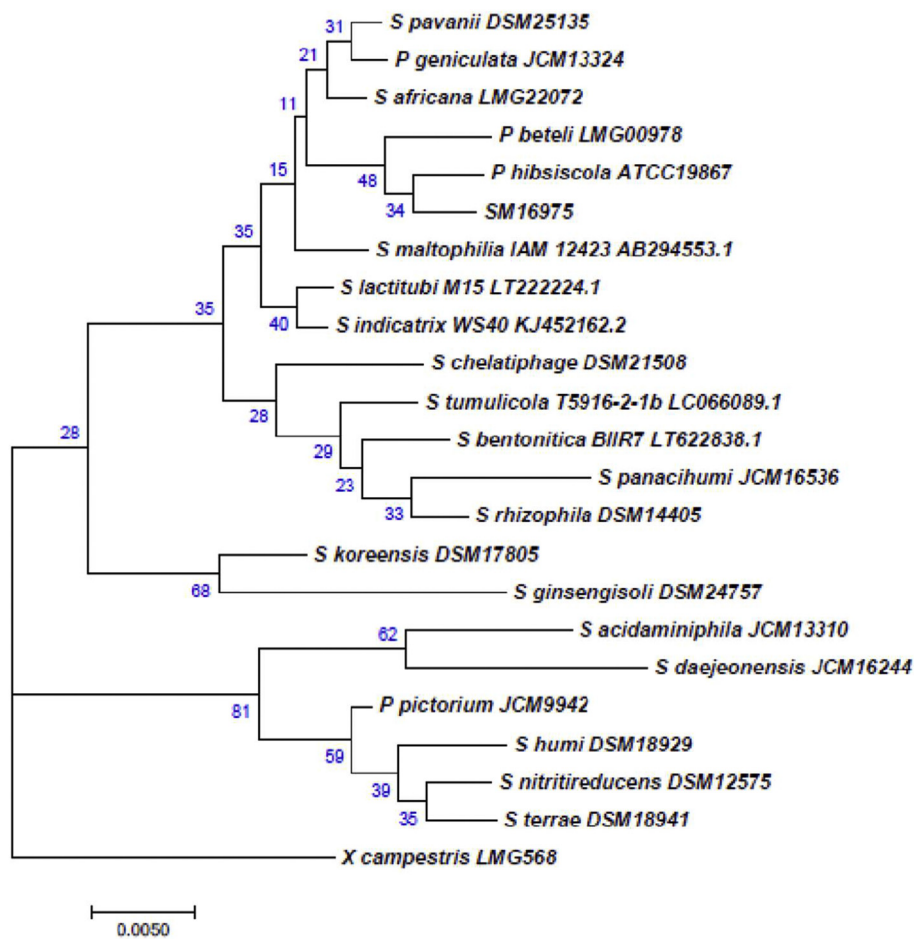


FIG. 1. Maximum-likelihood phylogenetic tree based on the alignment of complete 16s rRNA gene sequence of the Strain SM16975^T with type strains of species of the genus *Stenotrophomonas*, as well as species misclassified as members of the genus *Pseudomonas*.

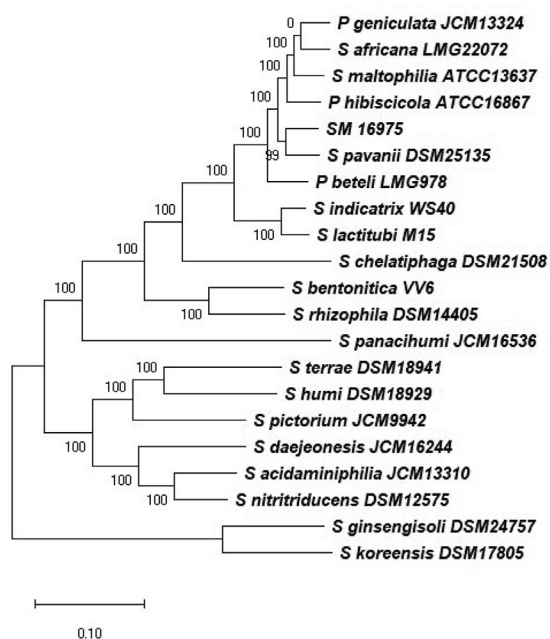


FIG. 2. Maximum-likelihood phylogenetic tree based on the alignment of the core genome of the Strain SM16975^T with the type strains of species of the genus *Stenotrophomonas*, as well as species misclassified as members of the genus *Pseudomonas*.

TABLE 3. Genome-based comparisons of SM16975^T and other type strains of members of the genus *Stenotrophomonas* and *Pseudomonas* (misclassified)

Reference strain	ANI (%)	OrthoANI (%)	AAI (%)	GDDC (%)
<i>Pseudomonas hibiscicola</i> ATCC 19867 ^T	91.98	92.15	94.49	46.4
<i>Stenotrophomonas pavanii</i> DSM 25135 ^T	91.96	92.22	94.61	46.6
<i>Pseudomonas geniculata</i> JCM 13324 ^T	91.13	91.48	93.91	44.2
<i>Stenotrophomonas maltophilia</i> ATCC 13637 ^T	91.11	91.37	94.07	44.3
<i>Stenotrophomonas africana</i> LMG22072 ^T	90.64	91.15	93.59	43.2
<i>Pseudomonas beteli</i> LMG 978 ^T	90.57	90.78	93.39	41.8
<i>Stenotrophomonas lactitubi</i> DSM 104152 ^T	86.49	87.1	88.29	32.1
<i>Stenotrophomonas indicatrix</i> DSM28278 ^T	86.42	86.95	88.55	31.9
<i>Stenotrophomonas chelatiphaga</i> DSM 21508 ^T	81.52	82.29	69.31	25.4
<i>Stenotrophomonas rhizophila</i> DSM 14405 ^T	81.11	91.64	79.49	24.5
<i>Stenotrophomonas bentonitica</i> LMG 29893 ^T	80.6	81.62	77.64	25
<i>Stenotrophomonas daejeonensis</i> JCM 16244 ^T	79.79	80.35	76.05	23.6
<i>Stenotrophomonas acidaminiphila</i> JCM 13310 ^T	79.49	80.28	75.81	23.8
<i>Stenotrophomonas pictorium</i> JCM 9942 ^T	78.56	79.21	76.03	22.6
<i>Stenotrophomonas panachumi</i> JCM 16536 ^T	77.95	78.89	75.53	22.3
<i>Stenotrophomonas terrae</i> DSM 18941 ^T	77.8	78.81	72.28	22.7
<i>Stenotrophomonas humi</i> DSM 18929 ^T	77.09	78.72	75.11	22.5
<i>Stenotrophomonas nitritireducens</i> DSM 12575 ^T	76.69	80.55	75.89	23.8
<i>Stenotrophomonas ginsengisoli</i> DSM 24757 ^T	76.05	77.16	70.47	21.2
<i>Stenotrophomonas koreensis</i> DSM 17805 ^T	76.01	76.71	70.07	21.3

Conclusion

Based on all the phenotypic, biochemical, and genomic tests performed on the bacterial strain, we conclude that strain SM16975^T belongs to the genus *Stenotrophomonas*. The taxonomic evidence from this study, such as the sequence similarity obtained using ANI, orthoANI, AAI, and dDDH were less than the defined cut-offs for species delineation. Therefore, SM16975^T is proposed as the type strain of the new species *Stenotrophomonas sepilia* sp. nov.

Nucleotide sequence accession number

The genome sequences were deposited in Genbank under accession number LXXZ00000000.

Deposit in culture collections

Strain SM16975^T was deposited in two different strain collections under the numbers (= JCM 32102; = KCTC 62052).

Transparency declaration

The authors declare that there are no conflicts of interest.

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Ethical statement

No experiments with humans or animals were carried out. The patient consent form is not required as the strain SM16975^T has been isolated as a part of routine diagnostic services.

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