GENOME-BASED RECLASSIFICATION OF *Stenotrophomonas maltophilia* 1800 AS *S. geniculata* AND GENOMIC INSIGHTS INTO ITS POTENTIAL CLINICAL AND ENVIRONMENTAL RELEVANCE

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Abstract. An *in silico* genomic analysis was performed on *Stenotrophomonas* sp. strain 1800 to clarify its taxonomic position and functional potential. The 16S rRNA gene sequence showed 100% similarity to *S. geniculata* ATCC 19374^T, and both strains shared identical genome size (4.8 Mb) and G+C content (66.2%). Genome-based comparisons revealed low relatedness between strain 1800 and *S. maltophilia* NBRC 14161^T (dDDH 47.8%, ANI 92.4%), whereas high dDDH (86.9%) and ANI (98.5%) values with *S. geniculata* ATCC 19374^T unambiguously support the reclassification of strain 1800 as *S. geniculata*. Functional annotation showed that core metabolic pathways dominate the genome, with 1568 genes assigned to subsystems, mainly involved in amino acid and protein metabolism. The CAZome represents 1.81% of predicted coding sequences, indicating notable carbohydrate-transforming capacity. Additionally, the genome encodes diverse virulence-, persistence-, and antimicrobial resistance-associated determinants, as well as genes involved in xenobiotic degradation and heavy metal resistance, suggesting both the clinical relevance and bioremediation potential of this strain.

Keywords: antimicrobial resistance; digital profiling; genomics; *Lysobacteraceae*; secondary metabolites.

INTRODUCTION

Stenotrophomonas is a member of the family *Lysobacteraceae* within the phylum *Pseudomonadota*. It comprises environmental, metabolically versatile, and frequently multidrug-resistant Gram-negative bacteria. According to the latest taxonomic updates, the genus *Stenotrophomonas* comprises 30 validly published and currently recognized species (https://lpsn.dsmz.de/; accessed on July 30, 2026). *Stenotrophomonas maltophilia*, is a non-fermenting bacillus recognized as an important opportunistic pathogen, particularly in immunocompromised patients. It is associated with infections such as pneumonia, bloodstream infections, and urinary tract infections, which are often accompanied by high morbidity and mortality rates [17, 18]. Its intrinsic and acquired resistance to antibiotics further complicates treatment, making it a difficult pathogen to control [45]. Beyond its clinical relevance, genome annotation identified predicted homologs of genes associated with the degradation of environmental pollutants and resistance to heavy metals. These findings suggest a potential genetic capacity for bioremediation-related functions in strain 1800; however, these predicted functions require experimental validation before any bioremediation capability can be confirmed [38, 42].

Accurate species identification within the genus *Stenotrophomonas* remains challenging because several species are phylogenetically closely related and exhibit highly similar phenotypic characteristics. Consequently, strains identified using conventional phenotypic or single-gene approaches may be incorrectly assigned to species. The widespread availability of whole-genome sequences, together with the application of genome-based taxonomic metrics such as average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH), has substantially improved bacterial systematics and has led to the reclassification of numerous misidentified strains. These genome-based approaches are now regarded as the reference standard for bacterial species delineation [5].

Strain 1800 was originally deposited as *Stenotrophomonas maltophilia* by Semai et al. [47]; however, its taxonomic assignment had not been re-evaluated using contemporary genome-based approaches. Given the close genetic relationships among members of the genus and the increasing recognition of taxonomic misclassifications in publicly available genome databases, the taxonomic status of this strain warranted re-evaluation. Therefore, the objectives of this study were to clarify the taxonomic position of strain 1800 through comprehensive genome-based analyses and to characterize its functional genomic repertoire, with particular emphasis on genes associated with environmental adaptation, persistence, virulence, antimicrobial resistance, and xenobiotic degradation. These analyses provide insights into the potential ecological and clinical relevance of this strain while recognizing that genomic predictions require experimental validation.

MATERIALS AND METHODS

Phylogenetic analysis

Phylogenetic analysis of 16S rRNA gene sequences was conducted for the type strains of *Stenotrophomonas* species with validly published names. Pairwise sequence similarities were calculated

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using the EzBioCloud database [54]. Multiple sequence alignments were generated with the CLUSTAL W algorithm using default parameters [51], and evolutionary analyses were carried out in MEGA11 [48]. Tree robustness was assessed by bootstrap analysis with 1000 replications [15], using the maximum likelihood method [14] with the Kimura two-parameter model and pairwise deletion [23].

Genomic dataset

The bacterium *Stenotrophomonas maltophilia* strain 1800 was isolated from the effluent of an industrial oil refinery in Algeria, and its genome was sequenced by Semai *et al.* [47]. The genomes of *S. maltophilia* strain 1800 and closely related species were retrieved from the NCBI GenBank repository (https://www.ncbi.nlm.nih.gov/data-hub/genome/; accessed on 07 September 2025). Genome annotation were performed using the NCBI server and the DDBJ Fast Annotation and Submission Tool (DFAST) (https://dfast.ddbj.nig.ac.jp/; [49]; accessed on November 29, 2025). Genome completeness and contamination values were obtained from the NCBI GenBank genome records, where they had been assessed using CheckM (v1.2.5), a lineage-specific marker gene-based software widely used for evaluating the quality of prokaryotic genomes.

Phylogenomic analysis

Digital DNA-DNA hybridization (dDDH) estimates, along with their corresponding confidence intervals, were generated using the Genome-to-Genome Distance Calculator (GGDC; http://ggdc.dsmz.de; accessed on November 13, 2025) with 100 replicates and the recommended default settings (Formula 2), which estimates dDDH values based on the d_4 genome distance and is the standard approach for draft bacterial genome comparisons [33, 34].

Average nucleotide identity (OrthoANI) values were computed for pairwise genome comparisons within the *Stenotrophomonas* genus using the EzGenome platform with the default parameters [29]. Phylogenomic reconstruction was carried out with the Type Strain Genome Server (TYGS; https://tygs.dsmz.de/; [35]). The phylogenetic tree was inferred with FastME [30] based on Genome Blast Distance Phylogeny (GBDP), midpoint-rooted [13], and visualized using PhyD3 [24]. Node support was evaluated from 100 pseudo-bootstrap replicates.

RASTtk server

Rapid Annotations using Subsystems Technology (RASTtk; pipeline v2.0; https://rast.nmpdr.org/; accessed on 11 September 2025), in conjunction with the Model SEED database, is a bioinformatics platform that provides fully automated bacterial genome annotation [7]. The platform predicts protein-coding sequences and assigns functional roles to annotated genes. These functions are further organized into subsystems, grouping genes into functional categories such as Clusters of Orthologous Groups (COGs). This framework enables the functional classification of the analyzed genes into COG subsystems and facilitates the assessment of gene distribution within each category.

Carbohydrate-Active Enzymes (CAZymes) and CAZome Analysis

The abundance and distribution of carbohydrate-active enzyme (CAZyme) families and their associated domains in the genome of *S. maltophilia* strain 1800 were analyzed using the dbCAN3 platform (https://bcb.unl.edu/dbCAN2/; [55]; accessed on December 19, 2025). This pipeline integrates three complementary annotation tools: DIAMOND, HMMER, and dbCAN_sub. DIAMOND performs protein-protein similarity searches against curated CAZyme reference databases, HMMER employs Hidden Markov Models (HMMs) to detect conserved CAZyme domains, and dbCAN_sub further refines CAZyme predictions by assigning proteins to specific subfamilies within CAZy families. Although DIAMOND provides fast and highly specific CAZyme annotation, the combined use of HMMER and dbCAN_sub enhances the sensitivity and robustness of CAZyme identification [53]. Accordingly, CAZyme annotation was conducted using DIAMOND (E-value $< 10^{-102}$), HMMER dbCAN (E-value $< 10^{-15}$, coverage > 0.35), and HMMER dbCAN-sub (E-value $< 10^{-15}$, coverage > 0.35), following the parameters recommended by Zhang *et al.* [55]. To ensure high-confidence annotations, a CAZyme family was considered present only when independently supported by all three methods, using a consensus-based scoring approach. Only genes meeting this stringent criterion were retained for downstream analyses.

Prediction of gene features and functional annotation using Prokka

Genome annotation was performed using Prokka (version 1.14.6) (https://usegalaxy.eu; accessed on December 3, 2025) with default parameters. Prokka is a widely used, high-accuracy annotation pipeline available through the Galaxy platform for the rapid annotation of bacterial genomes [46]. Prokka enables the identification of coding sequences and other genomic features within the analyzed genome and provides functional annotations based on curated databases. In this study, Prokka was used to annotate genes associated with virulence and persistence, as well as genes related to environmental adaptation and the bioremediation potential of *Stenotrophomonas* sp. 1800.

RESULTS

16S rRNA gene sequence-based phylogenetic analysis

The 16S rRNA gene sequence (1541 bp) retrieved *in silico* from the genome of *Stenotrophomonas* sp. strain 1800 was compared with corresponding sequences of type strains of the most closely related species obtained from the EzBioCloud server, and the results are presented in Figure 1. Phylogenetic analysis

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revealed that strain 1800 showed the highest 16S rRNA gene sequence similarity to *Stenotrophomonas geniculata* ATCC 19374^T (100%), followed by *S. riyadhensis* CFS3442^T (99.73%), *S. pavanii* DSM 25135^T and *S. sepilia* SM-16975^T (99.59%), *S. muris* DSM 28631^T (99.52%), *S. maltophilia* NBRC 14161^T (99.39%), *S. indicatrix* WS40^T (99.25%), *S. cyclobalanopsidis* TPQG1-4^T (99.03%), *S. lactitubi* M15^T (98.91%), and *S. tumulicola* T5916-2-1b^T (98.70%). Sequence similarities between strain 1800 and other members of the genus *Stenotrophomonas* were below 98.65%.

Genome fearures

Comparative genomic analyses were performed using the genome of *S. maltophilia* strain 1800 and those of the closely related type strains, whose genome assemblies and reference annotations are available in the NCBI Genome database. The genomic features described in this study are based on the annotations generated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and DFAST, which provided comprehensive structural annotation of the analyzed genomes. A comparative analysis of the genomic features of *S. maltophilia* strain 1800, *S. maltophilia* NBRC 14161^T, and *S. geniculata* ATCC 19374^T is presented in Table 1. Comparison of the studied strains revealed identical values for total genome length (4.8 Mb) and G+C content (66.2%) between strain 1800 and *S. geniculata* ATCC 19374^T. In addition, these two strains exhibited very similar numbers of genes and protein-coding sequences. These genomic similarities indicate that strain 1800 is more closely related to *S. geniculata* ATCC 19374^T than to *S. maltophilia* NBRC 14161^T.

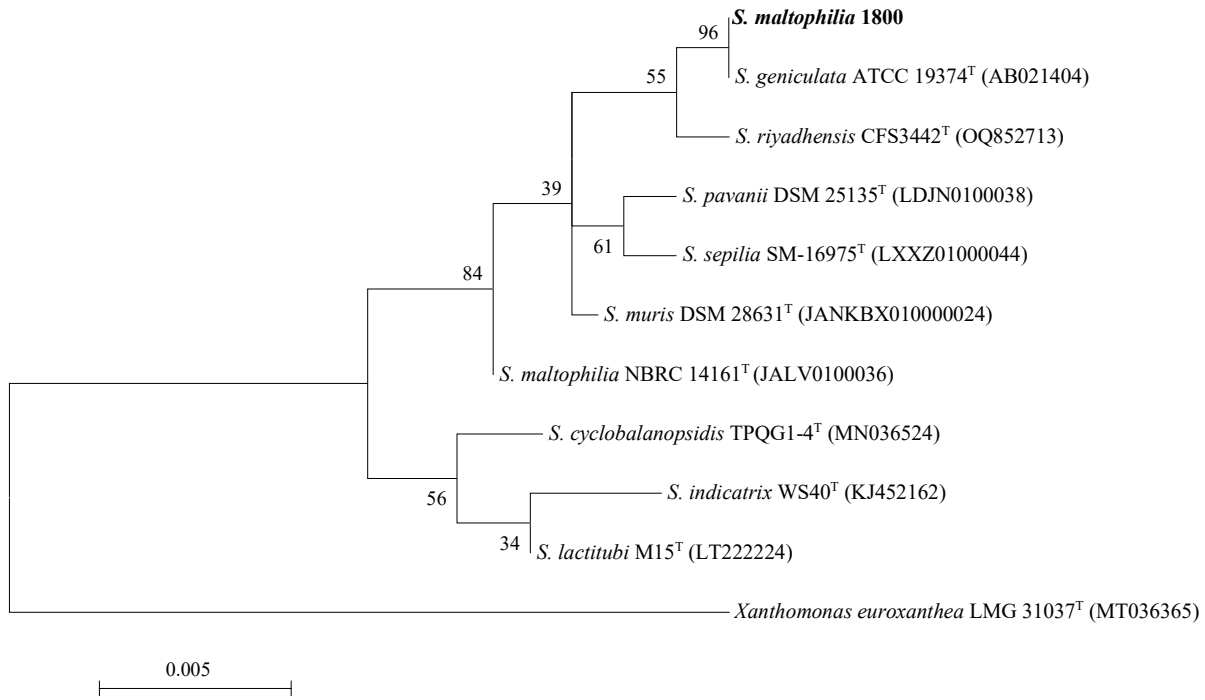

Figure. 1 Phylogenetic tree derived from nearly complete 16S rRNA gene sequences, showing relationships between the isolate *Stenotrophomonas maltophilia* 1800 and their phylogenetic neighbors. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model. Bootstrap values greater than 50% are indicated at nodes. Bar, 0.005 substitution per nucleotide position. *Xanthomonas euroxanthea* LMG 31037^T was used as the outgroup.

Table 1. Genome general features of *S. maltophilia* 1800, *S. maltophilia* NBRC 14161^T and *S. geniculata* ATCC 19374^T.

Characteristic	***S. maltophilia* 1800**	***S. maltophilia* NBRC 14161^T**	***S. geniculata* ATCC 19374^T**
GenBank assembly[a]	GCA_918593995.1	GCA_900186865.1	GCA_001431625.1
Total length (Mb)[a]	4.8	5.0	4.8
Number of contigs[a]	1	1	170
GC content (%)[b]	66.2	66.1	66.2
Gap ratio (%)[b]	0.0	0.0	0.0
Completeness (%)	98.26	98.44	98.52
Contamination (%)	1.19	1.08	0.53
Number of genes[a]	4798	4649	4496
Number of protein-coding genes[a]	4671	4555	4337
Number of tRNA[b]	75	80	70
Number of rRNA[b]	13	13	4
Number of pseudogenes[a]	31	54	57
Number of CRISPRS[b]	1	1	1

[a]features were obtained using the NCBI server; [b]features were obtained using the DFAST server.

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Phylogenomic analysis and Overall Genome Relatedness Indices (OGRI)

To clarify the taxonomic affiliation of strain 1800, a phylogenomic tree based on whole-genome sequences of the studied strain and closely related species was constructed using the TYGS Genome Server (Fig. 2). In parallel, Overall Genome Relatedness Indices (OGRI) analyses were performed (Table 2). Figure 2 presents a phylogenomic tree of the genus *Stenotrophomonas* inferred from whole-genome sequence data, with bootstrap support values indicated at the nodes. Strain 1800 clusters was closely related to the type strain of *S. geniculata*, forming a well-supported clade (bootstrap value = 97%). This close phylogenetic relationship strongly indicates that strain 1800 belongs to the species *S. geniculata*, as further illustrated by the shared species cluster (same color) in Figure 2. Moreover, the clear separation of strain 1800 from other closely related *Stenotrophomonas* species, supported by high bootstrap values, confirms its distinct species-level placement. The strong phylogenomic clustering, together with genome-based indices, further supports this classification. Specifically, the digital DNA-DNA hybridization (dDDH) value of 47.80% and the average nucleotide identity (ANI) of 92.44% between strain 1800 and *S. maltophilia* NBRC 14161^T indicate that these strains do not belong to the same species, as both values fall below the defined species delineation thresholds (70% for dDDH and 95% for ANI) [5, 6]. In contrast, high dDDH (86.90%) and ANI (98.51%) values were observed between strain 1800 and *S. geniculata* ATCC 19374^T, unambiguously confirming that strain 1800 belongs to *S. geniculata*.

Annotation of primary metabolites and core cellular functions

The functional categories and metabolic pathways described in this study are based on the subsystem annotations generated by RASTtk, which served as the reference framework for the functional characterization of strain 1800. Table 3 summarizes the distribution of predicted genes in *S. maltophilia* strain 1800 classified into functional subsystems using RASTtk and ranked in decreasing order according to gene count. The RAST subsystem-based annotation indicated that approximately 26% of the predicted coding sequences (CDSs) were assigned to functional subsystems, whereas the remaining CDSs were not classified within the RAST subsystem framework. Similar proportions of subsystem-assigned CDSs have been reported for other bacterial genomes, including *Planotetraspora* species (16%) [5], and *Actinopolyspora saharensis* (24%) [6], reflecting the inherent limitations of subsystem-based annotation and the incomplete functional characterization of many bacterial genes.

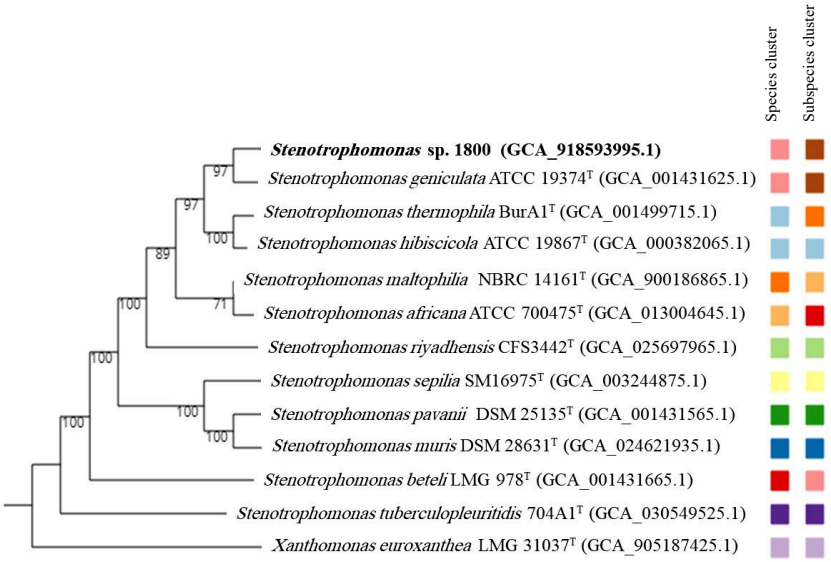

Figure 2. Phylogenomic tree based on whole-genome sequences generated using the TYGS Genome Server. The tree was inferred with FastME v2.1.6.1 [30] from Genome BLAST Distance Phylogeny (GBDP) distances calculated from whole-genome sequences. Branch lengths are scaled according to the GBDP distance formula *d5*. Numbers at the nodes indicate GBDP pseudo-bootstrap support values > 60%, based on 100 replicates. The tree was midpoint-rooted [13]. Strains sharing the same color belong to the same species cluster. *Xanthomonas euroxanthea* LMG 31037^T was used as the outgroup.

Table 2. dDDH%, and ANI% values between *S. maltophilia* 1800, S. *maltophilia* NBRC 14161^T and *S. geniculata* ATCC 19374^T.

	***S. maltophilia* 1800**		***S. maltophilia* NBRC 14161^T**		***S. geniculata* ATCC 19374^T**	
	dDDH, d_4 %	**OrthoANI %**	**dDDH, d_4 %**	**OrthoANI %**	**dDDH, d_4 %**	**OrthoANI %**
***S. maltophilia* 1800**	-	-	47.80	92.44	86.90	98.51
***S. maltophilia* NBRC 14161^T**	47.80	92.44	-	-	48.50	92.66
***S. geniculata* ATCC 19374^T**	86.90	98.50	48.50	92.66	-	-

dDDH similarities were calculated using Genome-to-Genome Distance Calculator (formula 2) webserver 3.0 (http:// ggdc. dmz. de).
The results were interpreted according to the recommended species delineation thresholds of dDDH ≥ 70% and OrthoANI ≥ 95%.
^{T} Indicates type strain.

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A total of 1568 genes were assigned to subsystems. The genome is dominated by genes involved in core metabolic and cellular functions. The largest functional category corresponds to amino acids and derivatives (253 genes), reflecting the organism's strong capacity for amino acid biosynthesis, degradation, and interconversion. This is followed by protein metabolism (197 genes), highlighting extensive machinery for protein synthesis, modification, folding, and degradation. Genes related to membrane transport (172 genes) and carbohydrate metabolism (161 genes) are also highly represented, indicating a high level of metabolic versatility and the ability to utilize and transport diverse substrates. The substantial number of genes associated with cofactors, vitamins, prosthetic groups, and pigments (128 genes) further supports the presence of complex enzymatic and redox systems. Energy generation and genetic information processing are well represented, with respiration (98 genes), DNA metabolism (83 genes), and RNA metabolism (39 genes), underscoring robust capabilities for growth, replication, and transcriptional regulation. In addition, fatty acids, lipids, and isoprenoids (66 genes) contribute to membrane biosynthesis and cellular homeostasis. Notably, genes involved in stress response (63 genes) and motility and chemotaxis (62 genes) are abundant, suggesting strong adaptive potential to fluctuating environmental conditions and the ability to actively respond to chemical gradients. Several subsystems are represented by a relatively small number of genes, including phosphorus metabolism (25 genes), cell wall and capsule (23 genes), regulation and cell signaling (22 genes), and metabolism of aromatic compounds (12 genes). Pathways related to iron acquisition and metabolism (9 genes) and secondary metabolism (8 genes) are limited but notable, as they may include specialized functions important for survival under nutrient-limited conditions. Only a few genes were assigned to sulfur metabolism (3 genes), nitrogen metabolism (6 genes), potassium metabolism (9 genes), and mobile genetic elements such as phages and transposons (2 genes). No genes were detected for nodulation, cell division and cell cycle, or photosynthesis, which is expected for a non-photosynthetic, free-living Gram-negative bacterium and reflects the limitations of subsystem-based annotation rather than the absence of essential division genes.

Annotation of carbohydrate-active enzymes (CAZymes) and CAZome analysis

The CAZome characterization presented in this study was based on the CAZyme annotations generated by the dbCAN3 pipeline, which served as the reference for identifying carbohydrate-active enzyme families and evaluating the carbohydrate metabolism potential of strain 1800. Table 4 presents the predicted carbohydrate-active enzymes (CAZymes) encoded by *Stenotrophomonas* sp. 1800, identified using a stringent approach that considered only those enzymes detected by all three dbCAN3 tools (DIAMOND, HMMER, and dbCAN_sub) and cross-referenced with the CAZy database. The analysis reveals a diverse repertoire of CAZymes, including auxiliary activities (AAs), carbohydrate-binding modules (CBMs), carbohydrate esterases (CEs), glycoside hydrolases (GHs), glycosyltransferases (GTs), and polysaccharide lyases (PLs).

Table 3. Distribution of predicted genes in *S. maltophilia* 1800 by subsystem category as annotated by RASTtk.

Subsystem features (Functional description)	Number of predicted genes
Nodulation	0
Amino acids and derivatives	253
Protein metabolism	197
Cofactors, vitamins, prosthetic groups, pigments	128
Carbohydrates	161
Nucleosides and nucleotides	56
DNA metabolism	83
RNA metabolism	39
Membrane transport	172
Sulfur metabolism	3
Virulence, disease, defense	50
Respiration	98
Cell wall and capsule	23
Fatty acids, lipids and isoprenoids	66
Nitrogen metabolism	6
Stress response	63
Miscellaneous	20
Regulation and cell signaling	22
Phosphorus metabolism	25
Metabolism of aromatic compounds	12
Potassium metabolism	9
Phages, prophages, transposable elements, plasmids	2
Cell division and cell cycle	0
Secondary metabolism	8
Dormancy and sporulation	1
Iron acquisition and metabolism	9
Motility and chemotaxis	62
Photosynthesis	0
Total number of genes	**1568**

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Carbohydrate-active enzymes (CAZymes) participate in the assembly, transformation, and degradation of carbohydrate molecules. Using the dbCAN3 platform, a total of 80 CAZyme-encoding genes were identified in the genome of the studied bacterium. These genes represent approximately 1.81% of the predicted protein-coding sequences and collectively define the organism's CAZome. Characterization of the CAZome provides valuable insight into the metabolic potential of the microorganism, including its capacity to process diverse carbohydrate substrates and to synthesize specific sugar-derived compounds.

Table 4. Predicted CAZymes in *Stenotrophomonas* sp. 1800 were compared using the CAZy database and dbCAN3 tools (DIAMOND, HMMER, and dbCAN_sub), considering only those CAZymes identified by all three tools.

Gene ID	Consensus CAZyme family
0	**1**
OU943334.1_2071	AA3
OU943334.1_4267	AA10+CBM73
OU943334.1_4268	AA10+CBM73
OU943334.1_2487	CBM0+GH13
OU943334.1_587	CBM12+GH18
OU943334.1_1944	CBM32
OU943334.1_2985	CBM32
OU943334.1_4207	CBM32+CBM51+GH31
OU943334.1_2486	CBM48+GH13
OU943334.1_2483	CBM48+GH13
OU943334.1_930	CBM50+GH23
OU943334.1_3162	CBM50+GH23
OU943334.1_2278	CBM91+GH43
OU943334.1_3681	CE4
OU943334.1_4435	CE4
OU943334.1_3022	CE4+GH153
OU943334.1_3741	CE9
OU943334.1_4212	CE9
OU943334.1_708	CE11
OU943334.1_2279	CE20
OU943334.1_2021	GH2
OU943334.1_377	GH3
OU943334.1_2277	GH3
OU943334.1_2282	GH3
OU943334.1_2624	GH3
OU943334.1_3266	GH3
OU943334.1_1275	GH5
OU943334.1_1116	GH13
OU943334.1_1121	GH13
OU943334.1_2367	GH13
OU943334.1_2484	GH13
OU943334.1_3470	GH15
OU943334.1_3115	GH18
OU943334.1_2696	GH19
OU943334.1_3747	GH20
OU943334.1_456	GH23
OU943334.1_1483	GH23
OU943334.1_3731	GH23
OU943334.1_2275	GH43
OU943334.1_2280	GH67
OU943334.1_2132	GH73
OU943334.1_2016	GH92
OU943334.1_2434	GH92
OU943334.1_3660	GH92
OU943334.1_1118	GH97
OU943334.1_117	GH102
OU943334.1_4205	GH109
OU943334.1_2989	GH144
OU943334.1_545	GT2
OU943334.1_656	GT2
OU943334.1_4312	GT2
OU943334.1_4352	GT2
OU943334.1_3023	GT2
OU943334.1_3360	GT2
OU943334.1_3960	GT2
OU943334.1_546	GT4
OU943334.1_907	GT4
OU943334.1_3141	GT4
OU943334.1_3658	GT4
OU943334.1_3961	GT4
OU943334.1_4198	GT4
OU943334.1_2488	GT5
OU943334.1_939	GT9
OU943334.1_1375	GT19
OU943334.1_3471	GT20
OU943334.1_4199	GT26
OU943334.1_702	GT28
OU943334.1_3655	GT30
OU943334.1_816	GT41
OU943334.1_3367	GT41
OU943334.1_3319	GT51
OU943334.1_3361	GT51
OU943334.1_3406	GT51
OU943334.1_3549	GT51
OU943334.1_3291	GT83
OU943334.1_3962	GT83
OU943334.1_3771	GT119
OU943334.1_1279	GT121
OU943334.1_2327	PL6
OU943334.1_2328	PL17
CAZYme gene	**80**
% CAZome	**1.81**

Only consensus CAZyme families independently identified by all three dbCAN3 annotation tools (DIAMOND, HMMER, and dbCAN_sub) are presented.

Virulence- and persistence-associated features in the genome of *Stenotrophomonas* sp. 1800

The genomic analyses presented in this study were based on the standardized genome annotations generated by Prokka, which provided reliable gene predictions and functional assignments for all downstream analyses. Table 5 summarizes the selected genes associated with virulence and persistence identified in the genome of *Stenotrophomonas* sp. 1800. The targeted genomic analysis of *Stenotrophomonas* sp. 1800 revealed a comprehensive arsenal of genes directly linked to virulence and environmental persistence. The genome encodes predicted homologs of multiple specialized systems involved in initial host interaction and surface colonization, including genes for the major fimbrial subunit SMF-1, CFA/I fimbriae, and a Type IV pilus biogenesis protein (PilQ), which collectively facilitate adhesion. Subsequent establishment is supported by genetic determinants for biofilm formation, such as a biofilm growth-associated repressor and key lipopolysaccharide (LPS) assembly (lptA) and export proteins, which are crucial for creating a protective extracellular matrix and maintaining outer membrane integrity. The genome encodes a sophisticated motility and chemotaxis apparatus, with flagellar biosynthesis (*fliG*, *flgB*) and signal transduction (*cheA*) genes, enabling directed movement toward favorable niches. Notably, the strain is equipped with multiple active secretion strategies, including complete Type II (T2SS), Type III (T3SS), and Type IV (T4SS)

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secretion systems, likely used to deliver effector proteins and toxins. Among these potential virulence factors are extracellular serine proteases and metalloproteases (e.g., LoiP), which can degrade host tissues, and potent toxins like the serine/threonine kinase HipA and the stress-resistance toxin PasT, which are implicated in inducing bacterial persistence. A defining feature is its formidable antimicrobial resistance profile, anchored by two clinically significant β-lactamases, the intrinsic metallo-β-lactamase L1 and the extended-spectrum beta-lactamase Toho-1, and a diversified suite of efflux pumps, including the RND systems MexA and MdtA, the MFS transporter NorM, and the colistin resistance-associated protein EmrA. Finally, the presence of a ferric uptake regulator (*Fur*) and multiple ferripyoverdine receptor genes indicates a highly efficient system for iron acquisition, a critical resource during infection.

Environmental role and bioremediation potential

Table 6 presents selected putative genes related to bioremediation identified in the genome of *Stenotrophomonas* sp. 1800 based on Prokka annotation. The genomic annotation of *Stenotrophomonas* sp. 1800 reveals a substantial and diverse genetic repertoire with high potential for bioremediation, characterized by specific enzymes for degradation and broad-spectrum efflux systems for detoxification. The genome encodes predicted homologs of enzymes associated with the catabolism of aromatic and halogenated compounds, including a toluene dioxygenase component, a haloalkane dehalogenase, and a 2-haloacrylate reductase, suggesting a potential genetic capacity for the initial degradation of hydrocarbons and organohalides. This degradative potential is significantly augmented by a suite of accessory enzymes for xenobiotic modification, including nitroreductases, epoxide hydrolases, and a remarkable variety of esterases and amidases (e.g., cocaine esterase, kynurenine formamidase, and omega-amidase), which can hydrolyze ester bonds in pesticides, plasticizers, and other complex organic pollutants. Crucially, the strain's survival in contaminated environments is supported by robust efflux and resistance mechanisms. It encodes complete organic solvent tolerance systems, such as the TtgBI toluene efflux pump and the AaeA aromatic acid efflux pump, which actively remove toxic hydrocarbons. Furthermore, it harbors an extensive and specialized arsenal for heavy metal resistance, including fully articulated systems for cobalt/zinc/cadmium (CzcABC), nickel/cobalt (CnrA), arsenic (Acr3 pump), mercury (MerA reductase and MerT transporter), chromate (*ChrR* regulator), and copper (multiple P-type ATPases and Cop proteins). This genomic inventory positions *Stenotrophomonas* sp. 1800 as a metabolically versatile bacterium equipped not only to transform a wide array of organic xenobiotics but also to withstand the concomitant stress from inorganic co-contaminants.

Table 5. Predicted homologs of virulence- and persistence-associated genes identified in the genome of *Stenotrophomonas* sp. 1800.

Locus tag	Gene (product)	General function (category)
OHBLNMGF_00671	Major fimbrial subunit SMF-1	Adhesion and colonization
OHBLNMGF_03622	CFA/I fimbrial subunit E	
OHBLNMGF_03612	Type IV pilus biogenesis/competence protein PilQ	
OHBLNMGF_02032	Biofilm growth-associated repressor	Biofilm and surface persistence
OHBLNMGF_01937	Lipopolysaccharide assembly protein A	
OHBLNMGF_01086	LPS export system protein LptA	
OHBLNMGF_02116	*RpfG* (c-di-GMP phosphodiesterase response regulator)	Regulation and signaling
OHBLNMGF_01441	Transcriptional regulator *SlyA*	
OHBLNMGF_02166	Flagellar motor switch protein FliG	Motility and chemotaxis
OHBLNMGF_02189	Flagellar hook/basal body protein FlgB	
OHBLNMGF_02139	Chemotaxis protein CheA	
OHBLNMGF_00094	Ser/Thr-protein kinase toxin HipA	Secreted factors: toxins/persistence
OHBLNMGF_01881	Persistence and stress-resistance toxin PasT	
OHBLNMGF_00605	Type II secretion system protein G	Secretion systems
OHBLNMGF_02625	Type III secretion system secretin	
OHBLNMGF_02758	Type IV secretion system protein VirB4	
OHBLNMGF_03938	Extracellular serine protease	Secreted/degradative enzymes and proteases
OHBLNMGF_00006	Metalloprotease LoiP	
OHBLNMGF_00948	ATP-dependent Clp protease subunit	
OHBLNMGF_02451	Metallo-beta-lactamase L1 type 3	Antimicrobial resistance and efflux
OHBLNMGF_03517	β-lactamases: Beta-lactamase Toho-1	
OHBLNMGF_02083	Multidrug resistance protein MexA	
OHBLNMGF_04133	Multidrug resistance protein MdtA	
OHBLNMGF_04048	Multidrug resistance protein NorM	
OHBLNMGF_03762	Colistin resistance protein EmrA	
OHBLNMGF_01884	Ferric uptake regulation protein (*Fur*)	Iron acquisition and homeostasis
OHBLNMGF_01208	Ferripyoverdine receptor	

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Table 6. Predicted bioremediation-related genes identified in the genome of *Stenotrophomonas* sp. 1800 based on Prokka annotation.

Gene (locus tag)	Genomic location	General function (broad category)
OHBLNMGF_01124	Aromatic hydrocarbon degradation (toluene dioxygenase component)	Aromatic and xenobiotic degradation (confirmed enzymes)
OHBLNMGF_03416	Dehalogenation (haloalkane dehalogenase)	
OHBLNMGF_02933	Dehalogenation/detoxification (2-haloacrylate reductase)	
OHBLNMGF_00806	Nitro-compound reduction (NAD(P)H nitroreductase)	
OHBLNMGF_03132	Xenobiotic detoxification (soluble epoxide hydrolase)	
OHBLNMGF_03921	Ester/organic pollutant hydrolysis (carboxylesterase)	
OHBLNMGF_00464	Ester/organic pollutant hydrolysis (esterase)	
OHBLNMGF_02669	Ester/organic pollutant hydrolysis (cocaine esterase; broad esterase activity)	
OHBLNMGF_01295	Detoxification of nitro-compounds (nitronate monooxygenase)	
OHBLNMGF_02776	Xenobiotic oxidation (tetracycline monooxygenase-like enzyme)	
OHBLNMGF_02679	Aromatic amino acid catabolism (kynurenine formamidase; xenobiotic-related amidase family)	
OHBLNMGF_03059	Amide detoxification / nitrogen scavenging (omega-amidase YafV)	
OHBLNMGF_03580	Aromatic solvent efflux (toluene efflux pump transporter TtgB)	Organic solvent tolerance / efflux systems (confirmed transporters)
OHBLNMGF_03761	Aromatic solvent efflux (outer membrane component TtgI)	
OHBLNMGF_01671	Aromatic acid efflux (p-hydroxybenzoate efflux pump AaeA)	
OHBLNMGF_02478	Heavy metal efflux (CzcA; Co/Zn/Cd RND transporter)	Heavy metal resistance and detoxification (confirmed systems)
OHBLNMGF_02479	Heavy metal efflux (CzcB; Co/Zn/Cd RND system)	
OHBLNMGF_03718	Nickel/cobalt resistance (CnrA)	
OHBLNMGF_00137	Arsenic resistance (Acr3 efflux pump)	
OHBLNMGF_02342	Arsenic resistance (arsenical pump membrane protein)	
OHBLNMGF_01640	Mercury detoxification (mercuric reductase MerA)	
OHBLNMGF_01642	Mercury transport (MerT)	
OHBLNMGF_02615	Mercury resistance regulation (*mer* operon regulator)	
OHBLNMGF_00458	Cadmium/cobalt/zinc resistance (H^+/K^+ antiporter-type metal transporter)	
OHBLNMGF_01581	Cadmium response (CadI)	
OHBLNMGF_01586	Chromate resistance (chromate transport protein)	
OHBLNMGF_02244	Chromate stress response (anti-sigma-E factor ChrR)	
OHBLNMGF_01573	Copper resistance (copper-exporting P-type ATPase)	
OHBLNMGF_02060	Copper resistance (copper-transporting P-type ATPase)	
OHBLNMGF_02471	Copper resistance (CopC)	
OHBLNMGF_02472	Copper resistance (CopD)	
OHBLNMGF_02058	Copper homeostasis (CutC)	

DISCUSSION

Several bacterial strains isolated from diverse Algerian environments have been described as novel taxa or reported to possess extremophilic or biotechnologically relevant properties [2, 4, 12, 26, 36, 43, 44, 50]. In the 16S rRNA gene-based phylogenetic tree (Fig. 1), strain 1800 clustered with *Stenotrophomonas geniculata* ATCC 19374^T, sharing 100% sequence similarity. However, because the 16S rRNA gene provides limited taxonomic resolution within the genus *Stenotrophomonas*, whole-genome analyses were performed. The phylogenomic tree, together with dDDH and OrthoANI analyses, demonstrated that strain 1800 does not belong to *S. maltophilia*, contrary to the classification reported by Semai et al. [47], but instead belongs to *S. geniculata*. This conclusion is strongly supported by the clustering of strain 1800 with the type strain of *S. geniculata* in the phylogenomic tree and by ANI and dDDH values exceeding the accepted species delineation thresholds. Furthermore, the phylogenomic analysis grouped *S. hibiscicola* ATCC 19867^T and S. *thermophila* BurA1^T within the same species cluster, consistent with the recent reclassification of *S. hibiscicola* as *S. thermophila* [8].

Stenotrophomonas geniculata is widely distributed across diverse environmental niches and is increasingly recognized for its potential clinical relevance. Environmental isolates of *S. geniculata* have been recovered from fresh produce and other non-clinical sources, suggesting that this species may serve as a reservoir of opportunistic pathogens at the interface between environmental and human-associated habitats [40]. Recent studies have shown that environmental isolates of *S. geniculata* exhibit robust growth at 37°C and form biofilms at levels comparable to those of clinical strains, suggesting the presence of traits associated with persistence and host colonization [40]. Collectively, these findings highlight the ecological adaptability of S. *geniculata* and emphasize the importance of monitoring environmental strains, as they may facilitate the dissemination of opportunistic bacteria through the food chain and provide a potential link between environmental and clinical settings.

Subsystem-based genome annotation of *Stenotrophomonas* sp. 1800 revealed a metabolically versatile organism enriched in genes related to core metabolism, transport systems, stress response, and motility, reflecting strong ecological adaptability. The identification of numerous predicted homologs of virulence-, disease-, and defense-associated genes suggests a genetic repertoire that may be consistent with an opportunistic lifestyle in clinical settings; however, their functional roles remain to be experimentally validated.

The CAZyme repertoire of strain 1800 indicates substantial enzymatic versatility for carbohydrate

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metabolism. Genome annotation identified multiple auxiliary activity (AA) enzymes involved in the oxidative cleavage of recalcitrant polysaccharides, together with carbohydrate-binding modules (CBMs), carbohydrate esterases (CEs), glycoside hydrolases (GHs), glycosyltransferases (GTs), and polysaccharide lyases (PLs). Collectively, these enzyme families are predicted to support polysaccharide degradation, biomass deconstruction, carbohydrate biosynthesis, and the utilization of structurally diverse glycans [19, 21, 25, 28, 32, 37]. The diversity of these predicted CAZymes suggests that strain 1800 possesses broad metabolic flexibility for carbohydrate turnover and may have potential for carbohydrate-based biotechnological applications, although these predicted functions require experimental validation. The proportion of CAZyme-encoding genes identified in strain 1800 is also consistent with the range reported for prokaryotic genomes, reflecting adaptation to different ecological niches and nutritional strategies [3, 5, 6].

The genome of strain 1800 encodes predicted homologs of genes associated with adhesion, biofilm formation, motility, iron acquisition, secretion systems, antimicrobial resistance, and persistence, suggesting a genetic repertoire that may support survival in diverse environments, including clinical settings. The coexistence of predicted adhesins, flagellar components, chemotaxis proteins, and biofilm-associated genes indicates the potential for surface colonization and biofilm development. Genome annotation also identified homologs of β-lactamase genes (*L1* and *Toho-1*) and multiple multidrug efflux systems, which may contribute to resistance against β-lactams and other antimicrobial classes. In addition, predicted homologs of secretion system components (T3SS and T4SS), proteases, and persistence-associated proteins, including *HipA*, suggest a repertoire potentially involved in host interaction and bacterial persistence. Collectively, these genomic features are consistent with the opportunistic nature of *Stenotrophomonas* species reported in previous studies; however, the expression, regulation, and biological functions of these predicted genes require experimental validation before their contribution to pathogenicity or antimicrobial resistance can be confirmed.

Biofilm formation is considered an important characteristic of *Stenotrophomonas* species because it promotes persistence in clinical environments and increases tolerance to antimicrobial agents [9]. Genome annotation of strain 1800 identified predicted homologs of genes associated with biofilm formation, consistent with previous reports describing the contribution of this trait to chronic infections. In addition, virulence-associated genes, including homologs related to extracellular protease production, were identified; however, as these findings are based solely on genome annotation, their expression and functional roles require experimental validation [11].

Stenotrophomonas species are intrinsically resistant to multiple antimicrobial agents through a combination of low outer-membrane permeability and chromosomally encoded resistance determinants, while additional acquired resistance genes may further contribute to multidrug resistance [10, 22]. Consistent with these reports, genome annotation of strain 1800 identified predicted homologs of genes associated with antimicrobial resistance, including β-lactamases and multidrug efflux systems. The genome also contains predicted homologs of genes involved in quorum sensing, motility, and biofilm formation, traits that have been associated with bacterial persistence and host interaction in *silico* species [1, 9]. However, these genomic predictions do not demonstrate gene expression or biological activity, and their functional significance requires experimental validation.

The genome encodes predicted homologs of genes associated with biofilm formation, motility, and quorum sensing, which are recognized virulence-associated traits that may contribute to the pathogenic potential of *S. maltophilia* [17, 39]. Genome annotation identified 50 predicted genes associated with virulence, disease, and defense functions. These genes have been reported in opportunistic *Stenotrophomonas* species and may represent a potential genetic basis for host interaction, antimicrobial resistance, or environmental competitiveness, although their functional roles in strain 1800 remain to be experimentally validated.

The bacterium *S. maltophilia* is recognized for its ability to form biofilms, contributing to persistence in clinical settings, particularly on indwelling medical devices [16, 17]. The species also exhibits resistance to multiple antimicrobial agents, including β-lactams, fluoroquinolones, and aminoglycosides, although trimethoprim / sulfamethoxazole remains the preferred treatment despite increasing resistance [39]. These characteristics are consistent with the predicted homologs of genes associated with biofilm formation and antimicrobial resistance identified in the genome of strain 1800. In addition to its clinical relevance, *S. maltophilia* has demonstrated considerable bioremediation potential, including hexavalent chromium reduction [41], cadmium tolerance [31], diesel degradation [27], and deltamethrin degradation [20]. These previous findings provide context for the predicted homologs of genes associated with xenobiotic transformation and heavy metal resistance identified in strain 1800; however, the expression and functional activity of these predicted genes require experimental validation.

The genomic repertoire of *Stenotrophomonas* sp. 1800 suggests a potential genetic basis for adaptation to chemically complex and polluted environments, such as industrial sites or hydrocarbon-contaminated soils. Genome annotation identified predicted homologs of genes associated with the degradation of aromatic compounds and haloalkanes, together with genes encoding broad-substrate enzymes, including esterases, amidases, and nitroreductases. This

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combination of predicted functions may indicate a complementary metabolic strategy in which specialized enzymes initiate the transformation of specific pollutants, while broad-substrate enzymes participate in the subsequent processing of intermediate metabolites. Such a genetic repertoire could provide metabolic versatility for coping with complex pollutant mixtures commonly encountered in contaminated environments. In addition, the genome encodes predicted homologs of genes associated with solvent tolerance and heavy metal resistance, including efflux systems for organic solvents and aromatic compounds, as well as genes related to regulated metal efflux (e.g., Czc and Acr3) and mercury detoxification (MerA). Collectively, these genomic features suggest a potential capacity for persistence in co-contaminated environments; however, their expression, physiological activity, and contribution to pollutant degradation or environmental adaptation remain to be experimentally validated.

The genome of strain 1800 contains predicted homologs of genes associated with xenobiotic transformation and heavy metal resistance, suggesting a genetic repertoire that may support survival in chemically complex environments. The coexistence of predicted catabolic and resistance-related genes is consistent with an adaptive strategy that could facilitate persistence under multiple environmental stresses; however, these predicted functions require experimental validation before any bioremediation capability can be confirmed. Previous studies have shown that *S. maltophilia* is capable of degrading a wide range of petroleum hydrocarbons, including n-alkanes (C8-C36), through enzymes encoded by genes such as *alkB* and *rubA* [52]. The isolation of strain 1800 from an oil refinery is consistent with this ecological context, although its hydrocarbon-degrading capacity remains to be experimentally demonstrated. The increasing interest in the environmental applications of *Stenotrophomonas* species is reflected by a marked rise in studies published between 2006 and 2021 [16]. In addition, several *Stenotrophomonas* strains have demonstrated the ability to remove heavy metals and radionuclides, including uranium and mercury, highlighting the environmental potential of this genus [41, 56]. Collectively, these reports provide context for the predicted genomic repertoire of strain 1800 but do not constitute evidence of its functional capabilities.

In summary, the *in silico* genomic characterization of *Stenotrophomonas* sp. strain 1800 enabled its precise taxonomic reclassification as S. *geniculata*. Genome annotation identified a diverse repertoire of predicted genes, including carbohydrate-active enzymes, homologs of genes associated with virulence and persistence, putative antimicrobial resistance determinants, and genes associated with xenobiotic and heavy metal transformation. The predicted antimicrobial resistance determinants should be interpreted alongside the intrinsic resistance mechanisms that are characteristic of *Stenotrophomonas*, such as its inherently low outer-membrane permeability and constitutively encoded efflux systems, rather than as evidence of acquired resistance or phenotypically confirmed antimicrobial resistance. Collectively, these genomic features suggest a genetic repertoire potentially relevant to both clinical and environmental contexts; however, the expression and functional significance of the predicted genes remain to be experimentally validated. It is important to clarify that the reported antimicrobial resistance genes represent putative homologs identified through *in silico* genome analysis and do not, by themselves, demonstrate phenotypic antimicrobial resistance. Overall, this study illustrates how genome-based analyses can improve taxonomic resolution and provide insights into the predicted functional repertoire of environmentally and clinically relevant bacteria.

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