

GENOME REPORT

Open Access



The complete genome sequence of a bile-isolated *Stenotrophomonas maltophilia* ZT1

Min Zhang¹, Lixiang Li², Hongwei Pan³ and Tao Zhou^{1*}

Abstract

Background: *Stenotrophomonas maltophilia* is one of the most frequently isolated opportunistic pathogens that can cause infections in humans. Many researches concerned the mechanism of antibiotic resistance displayed by *S. maltophilia*, however, the mechanism of its pathogenesis and its adaptation to special niches, such as bile, remain unclear.

Results: In this study, the *S. maltophilia* strain ZT1 was isolated from human bile. Its genome was sequenced and a circular chromosome of 4,391,471 bp was obtained with a GC content of 66.51%. There were 3962 protein-coding sequences, 7 rRNAs and 74 tRNAs in the chromosome. Compared with Virulence Factor Database, we identified more than 500 candidate virulence genes including genes encoding fimbrial assembly protein, enterobactin synthesis pathway proteins, efflux pumps, and the DNA and/or proteins secretion system in the genome of strain ZT1. Additionally, there were at least 22 genes related to bile adaptation, including *emrAB*, *acrRAB*, *galU*, *rfbC*, *tolC* and *mdtABC*.

Conclusions: This is the first study to reveal the whole genome sequence of the ZT1 strain of *S. maltophilia* isolated from human bile. We identified hundreds virulence factors and 22 bile adaptation-related genes in the genome of the *S. maltophilia* strain ZT1. Further comparative genomic analysis and functional verification would aid in understanding the pathogenesis and bile adaptation of *S. maltophilia*.

Background

Stenotrophomonas maltophilia is the third largest non-fermentative gram-negative bacillus, after *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [1]. It can cause opportunistic infections, especially in hospitalized and immunocompromised patients. In recent year, the number of clinical isolates of *S. maltophilia* has shown an upward trend [2]. Many clinical studies have found that *S. maltophilia* strains are resistant to several antibiotics, making its treatment challenging and infection life-threatening [3]. Currently, *S. maltophilia* is considered an

emerging multidrug-resistant, global, and opportunistic pathogen [4].

S. maltophilia is a common microbe widely distributed in water, soil, plant-associated habitats, and animal tissues [5]. *S. maltophilia* has been isolated from the sputum, secretion, urine, blood, wound drainage and cerebrospinal fluid of patients [1]. These studies demonstrate the high environmental adaptability of *S. maltophilia* [2]. Currently, there are more than 600 genome assemblies of *S. maltophilia* accessible in the NCBI database. Many genomic studies have focused on the genes related to antibiotic resistance and the virulence of *S. maltophilia* [6–8]. However, few studies have investigated the genetic mechanism for environmental adaptation of *S. maltophilia*.

S. maltophilia is also a common bacterium in bile [9]. However, no study has focused on the genome of

*Correspondence: zhoutao@sdu.edu.cn

¹ Department of Gastroenterology, Qilu Hospital, Shandong University, 107 Wenhua Road, Shandong 250012 Jinan, People's Republic of China
Full list of author information is available at the end of the article



© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

bile-isolated *S. maltophilia* strains. Here, we report the whole genome sequence of bile-isolated *S. maltophilia* strain ZT1. The bile adaptation and virulence-associated genes of the *S. maltophilia* strain ZT1 were also analyzed.

Methods

Strain isolation and characterization

A bile sample of a cholelithiasis patient was obtained for microbe culture and the *S. maltophilia* strain ZT1 was isolated on blood agar. This strain was then cultivated in brain–heart infusion broth for genomic DNA extraction under anaerobic conditions at 37 °C for 12 h. The 16 s rRNA sequence was obtained by PCR using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') and then sequenced. The obtained 16 s rRNA sequence was compared to the NCBI website using BLAST.

Genome sequencing and de novo assembly

A Bacteria DNA isolation kit (OMEGA Bio-Tek Inc., Norcross, GA, USA) was used to extract the genomic DNA from an overnight culture of the *S. maltophilia* strain ZT1. Subsequently, the quality of the genomic DNA was tested using a TBS-380 fluorometer (Turner BioSystems Inc., Sunnyvale, CA, USA). Then, a highly qualified DNA sample (OD_{260/280} = 1.8–2.0, total amount > 6 µg) was utilized for genome sequencing. According to the sequencing protocol, approximately 3 µg of genomic DNA was sequenced using an Illumina HiSeq Sequencer (Illumina Inc. San Diego, CA, USA) in the PE150 mode. Illumina pipeline CASAVA v1.8.2 (Illumina Inc. San Diego, CA, USA) was used for base calling to produce the raw sequencing data. Meanwhile, another 3 µg of genomic DNA was used to generate a 20 K template library which was then sequenced using the PacBio RS Platform (Pacific Biosciences of California, Inc., Menlo Park, CA, USA).

The Illumina sequence data were used to establish the complexity of the genome as well as correct the PacBio long reads, as described previously [10]. First, genome assembly was performed using ABySS with multiple-Kmer parameters and then the optimal results was obtained for the assembly [11]. After that, the long reads generated by PacBio were assembled using Canu (<https://github.com/marbl/canu>). Then, the remaining local inner gaps were filled using GapCloser [12]. Finally, we corrected the single-base polymorphisms for the final assembly results.

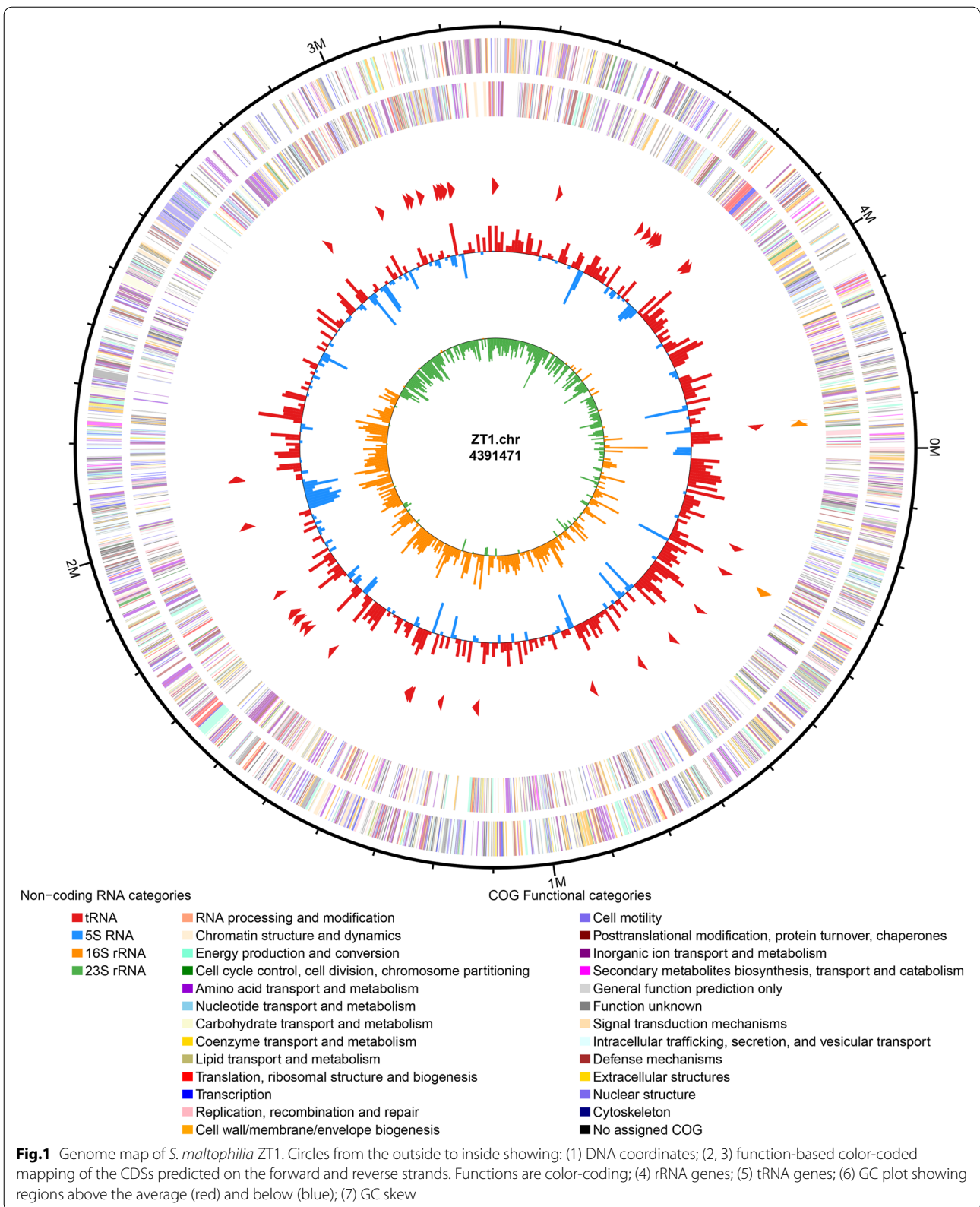
Gene annotation was performed using the automatic prokaryotic genome annotation pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The CGView Server was used to create the circular genomic map of strain ZT1 [13]. B, The phylogenetic analysis was

performed based on orthologous genes. First, OrthoMCL v2.0 was used to establish orthologous gene families with an E-value of 10⁻⁵ [14]. Second, the MUSCLE v3.8.31 software was used for multiple alignments [15]. Finally, phylogenetic analyses were performed using PhyML 3.0, based on maximum-likelihood (ML) methods. To calculate the bootstrap values, the GTR + G model was selected for ML analysis using 500 bootstrap replicates [16]. Several strains, including *S. maltophilia* NCTC10257 (LT906480.1), *S. maltophilia* SJTL3(CP029773.1), *S. maltophilia* Sm1 (GCA_001431665.1), *S. maltophilia* Sm2 (GCA_001651505.1), *S. maltophilia* Sm3 (GCA_001068765.1), *S. maltophilia* Sm4a (GCA_001069235.1), *S. maltophilia* Sm4b (GCA_000223885.1), *S. maltophilia* Sm5 (RAVG000000000), *S. maltophilia* Sm6 (GCA_002799015.1), *S. maltophilia* Sm7 (GCA_002798945.1), *S. maltophilia* Sm8 (RATO000000000), *S. maltophilia* Sm9 (GCA_001070945.1), *S. maltophilia* Sm10 (GCA_001297005.1), *S. maltophilia* Sm11 (GCA_002798885.1), *S. maltophilia* Sm12 (RAUV000000000), *S. maltophilia* Sm13 (ERR3300023), *S. maltophilia* Sm14 (GCA_002799245.1), *S. maltophilia* Sm15 (RATP000000000), *S. maltophilia* Sm16 (CP040439.1), *S. maltophilia* Sm17 (RAUD000000000), *S. maltophilia* Sm18 (GCA_002798925.1), *S. maltophilia* Sgn1 (GCA_002025605.1), *S. maltophilia* Sgn2 (GCA_002377295.1), *S. maltophilia* Sgn3 (GCA_000613205.1), *S. maltophilia* Sgn4 (GCA_001676435.1), *S. bentonitica* DSM103927 (GCA_013185915.1), *S. nitritireducens* 2001 (CP016756.1), *S. acidaminiphila* SUNE0 (CP019797.1), *S. rhizophila* QL-P4 (CP016294.1); *X. campestris* NEB122 (CP051651.1), *S. indicatrix* DAIF1 (CP037883.1), *S. lactitubi* As-6 (GCA_016921095.1), and *S. tumulicola* JCM 30961 (GCA_014117215.1) were selected for phylogenetic tree analysis. The putative virulence-related genes in the genome of the *S. maltophilia* strain ZT1 were identified using the Virulence Factor Database (VFDB) with a score > 80 and an e-value < 0.001 [17].

Results and discussion

General genome features of the *S. maltophilia* strain ZT1

A total of 2712.2 Mb data containing 18,081,966 raw reads were produced by the Illumina HiSeq Sequencer. After data filtering, 2611.8 Mb data containing 17,458,806 high-quality reads were obtained. Meanwhile, 115,046 (497.3 Mb) high-quality reads were produced by the Pac bio RS Platform with an average read length of 4322 bp. Sequence coverage was > 700-fold. Furthermore, we assembled these reads and obtained a high-quality genome with only one scaffold in this study (Fig. 1). This



complete genome is 4.19 Mb with a mean G+C content of 66.51%. After annotation, 3962 protein-coding sequences, seven rRNAs, and 74 tRNA genes were found in the chromosome of strain ZT1.

Phylogenetic analysis

The 16S rRNA gene sequence of strain ZT1 revealed the taxonomic status of strain ZT1 to be *S. maltophilia* by BLAST (data not shown). Phylogenetic analysis was carried out based on the whole genome DNA-sequence, and the results are shown in Fig. 2. A total of 24 type strains of *S. maltophilia*, including *S. maltophilia* NCTC10257 (LT906480.1), *S. maltophilia* SJTL3 (CP029773.1), *S. maltophilia* sm5 (RAVG00000000), were selected as standard. It was found that the strain ZT1 was most closely related to the *S. maltophilia* sm5 clade.

Identification of genes related to bile adaption

Bile acids are a group of steroids with high stability and biocompatibility. One of the major physiological roles is antimicrobial activity, as they have a structure similar to the antimicrobial peptides [18]. *S. maltophilia* strain ZT1 could live in bile, and which indicates an inherent

tolerance to high concentrations of bile. Genomic analysis of the *S. maltophilia* strain ZT1 showed the presence of 22 genes related to bile adaptation (Table 1). Half of them were genes encoding efflux pumps, including *emrAB*, *acrAB*, *tolC*, *mexAB*, *mdtABC*, and *porin F*. We also identified other genes, such as O-antigen biosynthesis-related (*rfbABC*), DNA binding protein (*hupB*), DNA mismatch repair protein (*mutS*), and zinc uptake regulation protein (*zur*) genes, which are related to bile adaption.

Analysis of virulence associated genes

S. maltophilia is a conditional pathogen and strain ZT1 was isolated from a patient with cholelithiasis. Therefore, analysis of putative virulence-associated genes in the genome of strain ZT1 was performed by aligning gene sequences to VFDB. The results indicated that more than 500 hundred virulence-associated genes were predicted in the genome of strain ZT1 (Additional file 1: Table S1). We found 88 gene related to flagellar biosynthesis in the genome of strain ZT1; additionally, there was a flagellar encoding gene island from ZT1002347 to ZT1002401. The virulence-associated fimbrial assembly

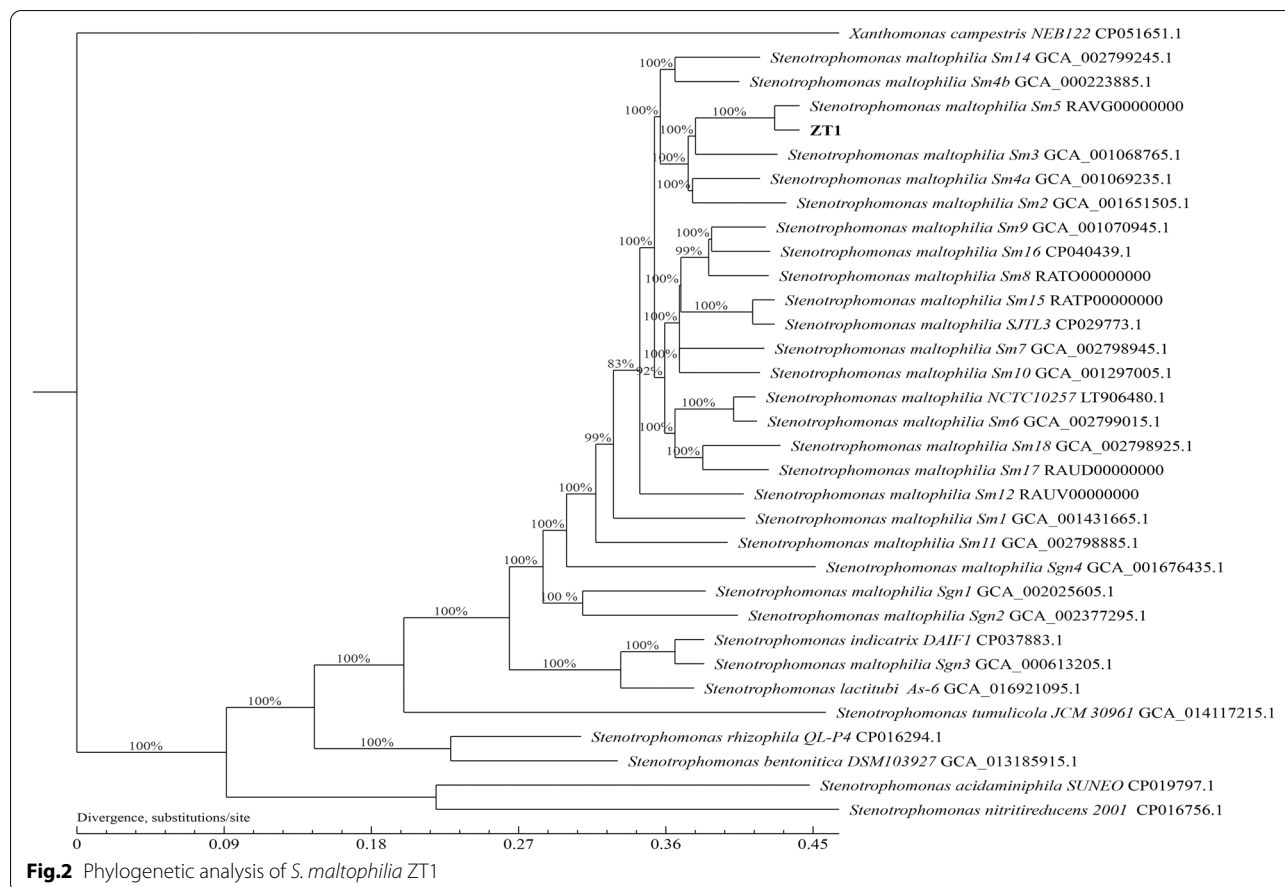


Table 1 The genes related to bile adaption in *S. maltophilia* ZT1

Gene ID	Swissprot_tophit_descrip	Swissprot top similarity %	KEGG gene name
ZT1002978	Multidrug export protein EmrB OS = Escherichia coli (strain K12)	49.4	emrB
ZT1002979	Multidrug export protein EmrA OS = Haemophilus influenzae (strain ATCC 51907)	45.2	emrA
ZT1000837	HTH-type transcriptional regulator AcrR OS = Shigella flexneri	31.4	acrR, smeT
ZT1000838	Multidrug efflux pump subunit AcrA OS = Escherichia coli (strain K12)	43.9	acrA, mexA
ZT1000839	Multidrug efflux pump subunit AcrB OS = Escherichia coli (strain K12)	64	acrB, mexB
ZT1000961	Outer membrane protein TolC OS = Vibrio cholerae serotype O1 (strain ATCC 39,315)	31.7	tolC
ZT1001690	Multidrug efflux pump subunit AcrB OS = Escherichia coli (strain K12)	50	acrB, mexB
ZT1001691	Multidrug efflux pump subunit AcrA OS = Escherichia coli (strain K12)	41.2	acrA, mexA
ZT1003728	O-antigen biosynthesis protein RfbC OS = Myxococcus xanthus	42.1	rfbC
ZT1003730	O-antigen export system ATP-binding protein RfbB OS = Myxococcus xanthus	39	ABC-LPSE.A
ZT1003731	O-antigen export system permease protein RfbA OS = Klebsiella pneumoniae	22	–
ZT1000559	Virulence transcriptional regulatory protein PhoP OS = Salmonella typhi	39.4	–
ZT1000233	Virulence transcriptional regulatory protein PhoP OS = Salmonella typhi	43.7	phoP
ZT1000632	Multidrug resistance protein MdtC OS = Enterobacter sp. (strain 638)	44.5	–
ZT1000633	Multidrug resistance protein MdtB OS = Xenorhabdus bovienii (strain SS-2004)	51.1	mdtB
ZT1000634	Multidrug resistance protein MdtA OS = Dickeya zeae (strain Ech586)	40.3	mdtA
ZT1003430	Outer membrane porin F OS = Pseudomonas aeruginosa (strain ATCC 15,692)	32	TC.OOP
ZT1003393	DNA-binding protein HU OS = Xylella fastidiosa (strain Temecula1 / ATCC 700,964)	76.4	hupB
ZT1002604	UTP-glucose-1-phosphate uridylyltransferase OS = Pseudomonas aeruginosa (strain PAO1)	46.5	galU, galF
ZT1002538	UDP-glucose 4-epimerase OS = Bacillus halodurans (strain ATCC BAA-125)	51.4	galE, GALE
ZT1003099	DNA mismatch repair protein MutS OS = Xanthomonas campestris (strain ATCC 33,913)	89.6	mutS
ZT1003056	Zinc uptake regulation protein OS = Shigella flexneri	37.6	zur

protein-encoding genes included pilB, pilQ, pilJ, pilT, pilC, pilU, pilM, pilD, flhA, mrkC, fauA, and flgI. Enterobactin is a virulence factor of *S. maltophilia* [19]. An entCEBFA operon (ZT1002005–ZT1002010) containing genes encoding for proteins of the enterobactin synthesis pathway as well as an enterobactin exporter encoding gene entS (ZT1001564) were found. The efflux pump-associated virulence factors are listed in Additional file 2: Table S2. Several RND family multidrug efflux pumps, NodT family efflux transporter, multidrug ABC transporter and other efflux pumps were found in the genome of strain ZT1, which may be related to antibiotic resistance in *S. maltophilia*. The DNA and/or protein secretion system is another type of virulence factor in *S. maltophilia* [20]. In the genome of strain ZT1, we found two independent type II secretion system operons, one containing 11 genes (xpsDNMLKJIHGFE; ZT1003667–ZT1003677) and the second contained at least another 11 gene (gspGKLMDEFJIHC, ZT1002072–ZT1002085). Some of these were identified as virulence factors, as shown in Additional file 3: Table S3. Meanwhile, a type IV secretion system operon was also found in the genome of strain ZT1, containing genes encoding for virB4, virB8, virB11. These virulence factors might contribute *S. maltophilia* infection and require further investigation.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13099-021-00456-y>.

Additional file 1. Table S1. The results indicated that more than 500 hundred virulence-associated genes were predicted in the genome of strain ZT1.

Additional file 2. Table S2. The efflux pump associated virulence factor in *S. maltophilia* ZT1 predicted by VFDB.

Additional file 3. Table S3. The secretion system associated virulence factor in *S. maltophilia* ZT1 predicted by VFDB.

Acknowledgements

We would like to thank Editage (www.editage.cn) for English language editing.

Authors' contributions

TZ and LXL designed the study; MZ and LXL isolated and identified the *S. maltophilia* strain ZT1; MZ and LXL analyzed data and wrote the manuscript; HWP revised the manuscript. LXL and TZ support this study the manuscript. All authors read and approved the final manuscript.

Funding

This research was supported by the Key Research and Development Program of Shandong Province (2019GHZ022 and 2019GSF108254).

Availability of data and materials

The completed genome sequence of *S. maltophilia* strain ZT1 has been deposited into GenBank database with accession number CP071784.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committees in Qilu hospital of Shandong University.

Competing of interests

The authors declare that they have no competing interests.

Author details

¹Department of Gastroenterology, Qilu Hospital, Shandong University, 107 Wenhua Road, Shandong 250012 Jinan, People's Republic of China.

²Key Laboratory of Translational Gastroenterology, Shandong University, 250012 Jinan, People's Republic of China. ³Department of Clinical Laboratory, Qilu Hospital, Shandong University, Shandong 250012 Jinan, China.

Received: 23 March 2021 Accepted: 6 October 2021

Published online: 28 October 2021

References

- Hu LF, Xu XH, Li HR, Gao LP, Chen X, Sun N, Liu YY, Ying HF, Li JB. Surveillance of antimicrobial susceptibility patterns among *Stenotrophomonas maltophilia* isolated in China during the 10-year period of 2005–2014. *J Chemother*. 2018;30(1):25–30.
- Adegoke AA, Stenstrom TA, Okoh AI. *Stenotrophomonas maltophilia* as an emerging ubiquitous pathogen: looking beyond contemporary antibiotic therapy. *Front Microbiol*. 2017;8:2276.
- Alsuhaibani M, Aljarbou A, Althawadi S, Alswed A, Al-Hajjar S. *Stenotrophomonas maltophilia* bacteremia in children: risk factors and mortality rate. *Antimicrob Resist Infect Control*. 2021;10(1):19.
- Brooke JS. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clin Microbiol Rev*. 2012;25(1):2–41.
- Pinski A, Zur J, Hasterok R, Hupert-Kocurek K. Comparative genomics of *Stenotrophomonas maltophilia* and *Stenotrophomonas rhizophila* revealed characteristic features of both species. *Int J Mol Sci*. 2020. <https://doi.org/10.3390/ijms21144922>.
- Yero D, Huedo P, Conchillo-Sole O, Martinez-Servat S, Mamat U, Coves X, Llanas F, Roca I, Vila J, Schaible UE, et al. Genetic variants of the DSF quorum sensing system in *Stenotrophomonas maltophilia* influence virulence and resistance phenotypes among genotypically diverse clinical isolates. *Front Microbiol*. 2020;11:1160.
- Han L, Zhang RM, Jia L, Bai SC, Liu XW, Wei R, Cui CY, Sun RY, Wang MG, Cai DT, et al. Diversity of L1/L2 genes and molecular epidemiology of high-level carbapenem resistance *Stenotrophomonas maltophilia* isolates from animal production environment in China. *Infect Genet Evol*. 2020;86:104531.
- Esposito A, Pompilio A, Bettua C, Crocetta V, Giacobazzi E, Fiscarelli E, Jousson O, Di Bonaventura G. Evolution of *Stenotrophomonas maltophilia* in cystic fibrosis lung over chronic infection: a genomic and phenotypic population study. *Front Microbiol*. 2017;8:1590.
- Kaya M, Bestas R, Bacalan F, Bacaksiz F, Arslan EG, Kaplan MA. Microbial profile and antibiotic sensitivity pattern in bile cultures from endoscopic retrograde cholangiography patients. *World J Gastroenterol*. 2012;18(27):3585–9.
- Yu T, Li L, Zhao Q, Wang P, Zuo X. Complete genome sequence of bile-isolated *Enterococcus avium* strain 352. *Gut Pathog*. 2019;11:16.
- Jackman SD, Vandervalk BP, Mohamadi H, Chu J, Yeo S, Hammond SA, Jahesh G, Khan H, Coombe L, Warren RL, et al. ABYSS 2.0: resource-efficient assembly of large genomes using a Bloom filter. *Genome Res*. 2017;27(5):768–77.
- Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *Gigascience*. 2012;1(1):18.
- Grant JR, Stothard P. The CGView server: a comparative genomics tool for circular genomes. *Nucleic Acids Res*. 2008. <https://doi.org/10.1093/nar/gkn179>.
- Li L, Stoeckert CJ Jr, Roos DS. OrthoMCL: identification of ortholog groups for eukaryotic genomes. *Genome Res*. 2003;13(9):2178–89.
- Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 2004;32(5):1792–7.
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol*. 2010;59(3):307–21.
- Chen L, Zheng D, Liu B, Yang J, Jin Q. VFDB 2016: hierarchical and refined dataset for big data analysis–10 years on. *Nucleic Acids Res*. 2016;44(D1):D694–697.
- di Gregorio MC, Cautela J, Galantini L. Physiology and physical chemistry of bile acids. *Int J Mol Sci*. 2021. <https://doi.org/10.3390/ijms22041780>.
- Kalidasan V, Joseph N, Kumar S, AwangHamat R, Neela VK. Iron and virulence in *Stenotrophomonas maltophilia*: all we know so far. *Front Cell Infect Microbiol*. 2018;8:401.
- Nas MY, White RC, DuMont AL, Lopez AE, Cianciotto NP. *Stenotrophomonas maltophilia* encodes a VirB/VirD4 type IV secretion system that modulates apoptosis in human cells and promotes competition against heterologous bacteria including *Pseudomonas aeruginosa*. *Infect Immun*. 2019. <https://doi.org/10.1128/IAI.00457-19>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

