Whole-Genome Sequencing and Analysis of Chryseobacterium arthrosphaerae from Rana nigromaculata

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Abstract

Chryseobacterium arthrosphaerae strain FS91703 was isolated from *Rana nigromaculata* in our previous study. To investigate the genomic characteristics, pathogenicity-related genes, antimicrobial resistance, and genetic evolutionary relationships of this strain, PacBio RS II and Illumina HiSeq 2000 platforms were used for the whole genome sequencing. The genome size of strain FS91703 was 5,435,691 bp and GC content was 37.78%. A total of 4,951 coding genes were predicted; 52 virulence factors and 94 virulence genes were identified, with the most relevant genes involved in the virulence factor of immune regulation. Analysis of antibiotic resistance genes revealed that strain FS91703 harbored 10 antibiotic resistance genes in 6 categories and 2 multidrug-resistant efflux pump genes, including adeG and farA. Strain FS91703 was sensitive to β-lactam combination drugs, cephem, monobactam and carbapenems, intermediately resistant to phenicol, and resistant to penicillin, aminoglycosides, tetracycline, fluoroquinolones, and folate pathway inhibitors. Phylogenetic and ANI analyses suggested that strain FS91703 had the closest genetic relationship to the *C. arthrosphaerae* species; 16S rRNA sequencing revealed that both species were on the same branch of the evolutionary tree; FS91703 and FDAARGOS 519 (*C. arthrosphaerae*) or ED882-96 (*C. arthrosphaerae*) had ANI values of 96.69% or 96.13%, respectively. Thus, *C. arthrosphaerae* (FS91703) is a multidrug-resistant and highly virulent bacterium, providing theoretical support for the etiology and disease control due to *C. arthrosphaerae* in *Rana nigromaculata*. The results of this study are presented in the following pages.

Background

The genus *Chryseobacterium* belongs to the family *Flavobacterium*. This genus consists of more than 100 species. These microorganisms are yellow-pigmented, non-spore-forming, non-motile, oxidase-positive, gram-negative, rod-shaped, and catalase-positive bacteria. They are abundant in water, soil, animals, and, plants. Among them, *C. meningoseptium*, *C. indogenes*, and *C. gellum* are considered as the most frequently isolated species from medical specimens [1]. The genus *Chryseobacterium* is an opportunistic pathogen that can cause meningitis, cellulitis, sepsis, and lower respiratory tract infections. The host range is very broad and includes humans and domestic animals [2, 3] as well as aquatic animals such as rainbow trout [4], snake eagles [5], sturgeon [6], and large pond turtles [1] *Chryseobacteriums spp*. are resistant to *cephem-*, *carbapenem-*, *aminoglycoside-* and *polymyxin* antibiotics. Infections caused by these microorganisms pose a serious threat.

C. arthrophaerae is a rare species of the genus *Chryseobacterium*, originally identified in 2010 from the feces of *Arthrophaerae magna* (pill millipedes) [7]. The ED882-96 strain of *C. arthrosphaerae* was identified in 2019 from a clinical patient with liver cirrhosis from the blood, and showed the resistance to all antibiotics tested; comparison with the virulence factor database (VFDB) identified 83 virulence factor homologues [8]. This was the first study involving virulence factor in *C. arthrophaerae*; Im et al. found a clinical patient with meningitis caused by *C. artherosphaerae* in 2020. The compound sulfamethoxazole was then proposed as a treatment for this disease [9] and the toxicity of this bacterium to humans was confirmed.

C. arthrosphaerae strain FS91703 was isolated from ascites fluid of *Rana nigromaculata*. In this study, the entire genome was sequenced and analyzed to determine the pathogenic mechanism, antimicrobial susceptibility, and evolutionary characteristics of this strain. The results provide theoretical support for the etiology and disease control caused by *C. arthrosphaerae* in *Rana nigromaculata*.

Results

Characterization of the genome of strain FS91703

The entire genome of strain FS91703 was sequenced and assembled, resulting in a final contig with 5,435,691 bp total length. The GC content was 37.78%, with 4,951 coding sequences. The coding genes was 4,754,943 bp length, with a 960 bp average length (87.48% of the total genome length). The interspersed repeats were 51 copies long, 3,208 bp (0.06% of the total genome length). Tandem repeats were 488 copies, 26,803 bp in length (0.49% of the total genome length). strain FS91703 contained 94 tRNA genes, 18 rRNA genes (including 6 each of 23S rRNA, 16S rRNA, and 5S rRNA), and 21 sRNA genes, with prophage 9, and the total length was 196 866 bp. The genome sequence and its annotation information were submitted to the NCBI database by accession number CP119767.

Table 1
Overview of genome function analysis of strain FS91703

Database	Gene number
NR	4 847
Swiss-Prot	2 388
KEGG	1 608
COG	3 041
GO	3 674
PHI	715
Pfam	14
VFDB	571
CARD	10
Secretory protein	658
T3SS	493
CAZY	145

Genome Functional Analysis Results

The FS91703 strain had 1,608, 3,674, 2,388, 3,041, and 4,847 genes in the KEGG, GO, Swiss-Prot, COG, and NR databases, respectively, the databases with the most annotated genes (see Table 1). The minimum number of annotated genes was 10.

NR of Strain FS91703

The gene sequence of strain FS91703 was converted to amino acid sequence and compared with the NR database, which showed that 4,847 genes were annotated in the NR database, of which *C. artherosphaerae* was the most frequently annotated, accounting for 76.48% (Fig. 1).

GO of Strain FS91703

The GO database was annotated with 3,674 genes. Cellular and metabolic processes in biological processes were the highest gene enrichment for two pathways with 1,376 and 1,393 genes, respectively. Cells and cell parts in cellular organization were the highest gene enrichment for the two pathways with 1,352 and 1,344 genes, respectively. Binding and catalytic activity in molecular function were the highest gene enrichment for the two pathways with 1,317 and 1,603 genes, respectively (Fig. 2).

A total of 1,608 orthologous protein-coding genes were matched to 39 KEGG metabolic pathways. The pathways with the greatest number of genes were amino acid metabolism, carbohydrate metabolism, metabolism of cofactors and vitamin, which are necessary to maintain bacterial metabolism (Fig. 3).

There were 3,041 genes annotated in the COG analysis. These were classified into 20 categories, C-V, according to function. The annotated functions mainly covered the pathways of biosynthesis of cell wall, membrane, and envelope, metabolism, amino acid transport, transcription (Fig. 4). The results were similar to those of the KEGG metabolic pathway analysis. Many genes were found to be involved in metabolic processes that sustain basic bacterial life.

PHI of FS91703 strain

The PHI database has annotated 798 genes that interact with animal hosts. Of these, 436, 90, and 49 genes were found to decrease, lose, or increase bacterial virulence after mutation. In the process of infecting the host, pathogenic bacteria secrete a series of effectors, which play an essential role in the interaction between the host and the pathogenic bacteria. The ability of effectors to effectively control the host is key to successful colony formation of pathogenic bacteria. Eight genes encoding effectors have been annotated in the PHI database, including *clpV5* (3), *lpdA* (2), *mgtC* (2) and *ipx10*. This gene was annotated. Studies have shown that deletion of the *clpV* gene in pathogenic E. coli reduces the expression of type I pili, affecting the ability of the bacteria to attach and invade, and reducing the pathogenicity of the strain [10].

VFDB and CARD of FS91703 strain

Virulence factors are grouped into 14 categories according to their functions; regulation, antimicrobial activity/competitive advantage, post-translational modifications, stress survival, nutritional/metabolic factors, biofilms, immune modulation, exoenzymes, exotoxins, motility, effector delivery system, invasion,

adhesion, and others. In this study, 52 virulence factors encoded by 94 genes were identified in the VFDB database. The virulence factors with the highest number of annotated genes were immune modulators, stress survival factors, and nutritional/metabolic factors, with 38, 19, and 12 genes annotated, respectively. Immune modulatory factors were mainly capsular, LPS O-antigen, LPS (lipopolysaccharide), and LOS (lipopolysaccharide). Among these factors, genes encoding capsules were the most abundant, including 9 genes such as *capK/L/5H*, *ndk*, *upps*, and *fnlA*. Stress resistance factors were mainly catalase, urease, ClpC, and MsrAB. Virulence factors associated with the effector secretory system were types III, IV, and VI. Of these, the most common virulence factors associated with the type III secretory system were T3SS, Psyringae TTSS effectors, and Mxi-Spa TTSS effectors regulated by MxiE. In the nutrient metabolism system, many factors related to iron uptake were found, including heme biosynthesis, aerobactin siderophores, and desferrioxamine. Four bacterial toxins were predicted: colibactin, β-hemolysin, the phytotoxin coronatine, and phosphatidylinositol-specific phospholipase C (PI-PLC). In addition, several drug efflux pump systems were annotated, including AdeFGH and FarAB.

The CARD database was used for the annotation of the total of 10 genes for antibiotic resistance in strain FS91703. They are *sul2* (2), *IND-14*, *catB2*, *catB6*, *catB8*, *tetX* (2), *dfrE*, and *streptomycetes*, and are resistant to folate pathway inhibitors (*sul2/dfrE*), penicillin (*IND-14*), cephem (*IND-14*), carbapenems (*IND-14*), phenol (*catB2*, *catB6*, *catB8*), tetracycline (*tetX*), and ercomycin (*streptomyces*). These correspond to the following antibiotics.

Genome-wide map of strain FS91703

Based on basic genome sequence information, gene prediction results, non-coding RNA prediction results, and bioinformatics analysis results, we drew a whole genome map of this fungus (Fig. 5).

Antimicrobial activity of strain FS91703

The antimicrobial susceptibilities of strain FS91703 and MICs are shown in Table 2. Strain FS91703 was susceptible to β-lactam combination agents, cephems, monobactams, and carbapenems, and was intermediate to phenicol. It was also resistant to all antimicrobial agents tested, including folate pathway inhibitors, fluoroquinolones, tetracyclines, aminoglycosides, and penicillin.

Table 2
Concentration of minimum growth inhibitory and antimicrobial susceptibility of *C. arthrosphaerae* FS91703.

Antibiotics Group	Antibiotics	MIC	Interpretation
Penicillin	Piperacillin	≥ 128	R
β-lactam Combination Agents	Piperacillin-tazobactam	≤ 16/4	R
Cephems	Ceftazidime	≤ 8	S
	Cefepime	≤ 8	S
Monobactams	Aztreonam	≤ 8	S
Carbapenems	Imipenem	≤ 4	S
	Meropenem	≤ 4	S
Aminoglycosides	Gentamicin	≥ 16	R
	Tobramycin	≥ 16	R
	Amikacin	≥ 64	R
Tetracyclines	Tetracycline	≥ 16	R
	Minocycline	≥ 16	R
Fluoroquinolones	Cipofloxacin	≥ 4	R
	Levofloxacin	≥ 8	R
	Lomefloxactn	≥ 8	R
Inhibitors of folate pathway	Trimethoprim-sulfmethoxazole	≥ 4/76	R
Phenicol	Chloramphenicol	16	I
Note: S-Sensitive, I-Intermediary, R-Resistant			

Phylogenetic relationships with 16S rRNA

The 16S rRNA of strain FS91703 was 1406 base pairs and was submitted to GenBank (accession number: ON573338). The 16S rRNA of strain FS91703 was compared with those of other bacteria registered in GenBank, and a phylogenetic tree was constructed by selecting species with high similarity (Fig. 6). Strain FS91703 belonged to the same branch as *C. arthrosphaerae* (CC-VM-7, FDAARGOS 519, and ED882-96 strains). The 16S rRNA of strain FS91703 was more than 99.85% identical to the 16S rRNA of the above three strains. These results suggest that strain FS91703 is one of the *C. arthrosphaerae* strains.

Average nucleotide identity (ANI) analysis

FS91703, two strains of *C. arthrosphaerae*, and 21 other *Chryseobacterium* species were analyzed by ANI. Intercomparison of FS91703 with FDAARGOS 519 and ED882-96 strains of *C. arthrosphaerae* showed ANI values of 96.69 and 96.13%, respectively; the ANI values between strain FS91703 and other strains of the genus *Chryseobacterium* were less than 85% (Fig. 7). The interspecies identification criteria with 95% ANI value confirmed that strain FS91703 is *C. arthrosphaerae*.

Discussion

As of March 1, 2023, the NCBI database uploaded the complete whole genome sequences of 11 *C. arthrosphaerae* species. However, studies on the biological characteristics of *C. arthrosphaerae* are still rare. Only the genomic function of *C. arthrosphaerae* strain ED882-96, collected from the blood of a clinical patient, has been analyzed. Further studies on the host range, pathogenicity, virulence, and antibiotic screening of *C. arthrosphaerae* are urgently needed.

Virulence factors are important factors for bacterial virulence; 94 virulence genes were annotated in strain FS91703 and 83 in strain ED882-96 by the VFDB database. Of these, 47 virulence genes encoding virulence factors such as LPS, capsule, effector delivery systems (types I, III, IV, and VI), colibactin, type IV pili, and polar flagella were identical in both strains. In addition, strain FS91703 had 47 unique virulence genes compared to strain ED882-96. Among them, 14 genes were involved in virulence factors for stress survival, including catalase, SodB, MsrAB, and ClpP. MsrAB catalyzes the reduction of protein-bound and free methionine sulfoxide (MetSO) to Met and repairs oxidized proteins. It is an enzyme with the ability to repair oxidized proteins. Catalase and SodB are important for intracellular survival and transmission, and this type of virulence factor makes microbes more resistant to oxidative stress [11–13]. Our results show that strain FS91703 has a strong resistance. There is also a difference in virulence between strain FS91703 and strain ED882-96.

The ED882-96 and FS91703 strains have different antibiotic resistance genes, which is reflected in their different antibiotic susceptibilities: the ED882-96 strain is resistant to penicillin, aminoglycosides, tetracycline, fluoroquinolones, and folate pathway inhibitors, whereas the FS91703 strain is susceptible to β-lactam/β-lactamase inhibitor combinations, cephalosporins, monocyclic lactams, and carbapenem β-lactams. Im et al. also found that C. artherosphaerae strains with human meningitis were sensitive to ciprofloxacin (fluoroquinolone), minocycline (tetracycline), tetracycline, and compound sulfamethoxazole (folate pathway inhibitor) and cured their cases with Compound sulfamethoxazole [9]. This indicates that there are differences in antibiotic resistance among the same bacterial species due to regional and host differences. In practice, antibiotic selection should be reasonably guided by antibiotic susceptibility results. The FS91703 strain also harbors genes such as adeG and farA, which encode multiple drug efflux pumps, AdeFGH and FarAB, respectively. Many studies have shown that drug efflux pumps can prevent drug accumulation in bacteria, creating internal bacterial resistance to toxic compounds such as antibiotics, disinfectants, detergents, and dyes. This is one of the main mechanisms of multidrug resistance in bacteria: the AdeFGH efflux pump can efflux almost all antibiotics [14]; the FarAB efflux pump can efflux long-chain fatty acids and some fat-soluble molecules [15]; and the FGH efflux pump

can efflux a number of lipids [16]. No fluoroquinolone resistance genes were detected in this study. However, strain FS91703 was resistant to fluoroquinolones such as cipofloxacin, levofloxacin, and lomefloxactn. These results suggest that the drug efflux pump may play an important role in the resistance of strain FS91703 to fluoroquinolones.

Strain FS91703 and strain ED882-96 were on the same branch of the phylogenetic tree according to 16S rRNA sequencing, with an ANI value of 96.13% for both. This result suggests that the relationship between the two strains is relatively close; further studies are needed to determine whether strain FS91703 can infect humans.

Here, we obtained whole genome information on *C. arthrosphaerae* FS91703 strain from *Rana nigromaculata* for the first time. Whole genome analysis revealed that this strain is highly virulent and multidrug-resistant, with many differences in virulence factors and drug resistance compared to strain *C. arthrosphaerae* ED882-96. This study adds new information to the genome database of this organism, enriches the host type, and provides a theoretical reference for subsequent studies.

Methods

Strain FS91703

Recently deceased black-spotted frogs with ascites fluid were collected from a farm in Zhejiang Province of China. The ascites fluid was patterned on LB plates under sterile conditions. The plates were placed in a incubator at 37℃ for 48 hours. A yellow colony of bacteria was isolated. It was named strain FS91703 and maintained at -80°C in glycerol stock.

Whole genome sequencing and assembly

The genome sequencing of strain FS91703 was performed by the the Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA) and PacBio RS II platform (Pacific Biosciences, Menlo Park, CA, USA). The sequence data (Subreads) were determined using the Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA). Sequence data (Subreads) were self-corrected and assembled into genomes using Falcon [17]. Consistency sequences (Consumus) were first obtained based on the Overlap-Layout-Consense algorithm; GenomicConsumes was used and subreads were corrected again based on the arrow algorithm. Then, using the Corrected Subreads corrected with spai (single pass read accuracy improver) as auxiliary data, the assembled consensus sequences (Corrected Consumes) were cycled [18], and finally the cycled bacterial genome (Genome) was obtained.

Bioinformatics Analysis

Genomic component predictions included coding gene, non-coding RNA, repeat sequence, and prophage predictions. Prokaryotic Dynamic Programming Genetic Algorithm (Prodigalv, 2.6.3), RepeatMask (v4.0.7) [19], PhiSpy (v2.3) [20] software were used to predict coding genes, repeat sequences, and prophages,

respectively. Genomic components of rRNA, sRNA, and TRNA were predicted using RNAmmer (v1.2), Rfam (v10.0), and tRNAscan-SE (v1.3.1) software, respectively [21–23].

Genome annotation and functional analysis

Common Function Database Annotation

Functional genomic analysis was performed by the on-line software, such as Cluster of orthologous groups of proteins (COG, https://www.ncbi.nlm.nih.gov/COG/), Non-Redundant Protein Database (NR, https://www.ncbi.nlm.nih.gov/), Swiss-Prot (http://www.uniprot.org/), Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/pathway.html) [24], Carbohydrate-Active enZYmes Database (CAZy, http://www.cazy.org) [25], Gene Ontology (GO), Pfam(http://pfam.xfam.org/)database, and evolutionary genealogy of genes (eggNOG, http://eggnog.embl.de/). The annotation of the KEGG, eggNOG, Swissprot, GO, COG, and NR databases was performed for the comparison with the DIAMOND [26] software, and the proteins with the highest sequence similarity with annotations e < 1e-5 were selected to gain the information of functional annotation. HMMER [27] software and protein family models were used to compare the annotations in the Pfam and CAZy. The annotations were compared and the protein families with the highest scores were screened.

Other Function Database Annotation

The Pathogen host interactions (PHI, http://www.phi-base.org/) database [28] was used to analyze pathogen-host interactions. Pathogenicity factors were predicted using the virulence factors of pathogenic bacteria (VFDB, http://www.mgc.ac.cn/VFs/main.htm) [29].The comprehensive antibiotic research database (CARD, https://card.mcmaster.ca/) was used for genes related to antibiotic resistance [30].

Genome-wide map of Strain FS91703

The circos (v0.69) software was used for the circular genome to create a graphical map [31].

Antimicrobial Susceptibility

The microdilution method was used for the determination of minimum inhibitory concentration (MIC). Guidelines of Clinical and Laboratory Stanards Institute (CLSI) were used for the interpretation of susceptibility of antimicrobial based of the criteria for other non-Enterobacteriaceae [32].

Phylogenetic tree by sequencing of 16S rRNA genes

The sequencing of 16S rRNA gene of strain FS91703 was obtained from the whole genome sequencing results, BLAST comparison was performed on NCBI, homologous sequences with high similarity were selected. The Neighbor-Joining method [33] in MEGA 7.0 software was use for constructing the phylogenetic tree [34].

Analysis of Average Nucleotide Identity (ANI)

Average nucleotide identity (ANI) is one of the key indicators to identify genetic distance between bacterial genomes and can reflect evolutionary distance OrthoANI was used to calculate ANI values [35]. In general, 95% of the ANI value is considered the criterion for separating the same species [36]. The heat maps were generated by CIMminer (https://discover.nci.nih.gov/cimminer).

Declarations

Ethics approval and consent to participate

All experiments were conducted in accordance with relevant guidelines and regulations, and all protocols were reviewed and approved by the Institution Animal Care and Use Committee (ACUC) of Zhejiang Academy of Agricultural Sciences (2019ZAASLA57).

Consent for publication

Not applicable.

Availability of data and materials

The complete data set was submitted to the National Biotechnology Information Center (NCBI) database with the accession number CP119767.

Data has been uploaded to the website https://www.ncbi.nlm.nih.gov/bioproject/PRJNA943546.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XP designed the study and revised the manuscript. LZ performed experiments, analyzed data, and wrote the manuscript. HL and XL assisted in analyzing the data. YS participated in the design of the experiment. And XY assisted in performing experimentss. All authors have read and approved the final manuscript.

References

1. Cai J, Wu JY, Pan YX, Wang M, Qi Y. Isolation, Identificaiton and drug resistance analysis of *Chryseobacterium* sp. from *Pseudosciaena crocea*. China Animal Husbandry and Veterinary

Medicine. 2022;49(3):1135-43.

2. Hu W J, Song Y H, Qin J C, Shi K, Yu WH, Liu Y, et al. Study on the change law and pathogenicity of postpartum bovine intrauterine flora. Hubei Agricultural Sciences. 2014; 53(7): 4115-9.
3. Xie CB, Luo JR, Zhao QM, Yu H. The study on drug resistance and resistant genotype of Chryseobacterium indologens. Clin Lab Med. 2019; 16(1):20-6.
4. Jeong JJ, Lee YJ, Pathirajad M, Pathiraja B, Choi Ki, Deok Kim. Draft genome sequences of *Chryseobacterium lactis* NCTC 11309 T islateed from milk, *Chryseobacterium oncorphychi* 701B-08 T from rainbow trout, and *Chryseobactium viscerum* 687B-08 T from diseased fish. Genome Announcements. 2018; 6(26): e00628-18.
5. Wang XY, Han YN, Jin S. Identification of indole-producing *Aureobacillus* from *Ophiocephalus argus and* analysis of characteristics of extracellular products. Acta Hydrobiol Sin. 2016; 40(3): 641-6.
6. He SX, Ma GQ, Niu WJ. Study on pathogenicity of a pathogenic bacterium from sturgeon in northern China. J Agr Sci Tech. 2019; 21(4): 96-103.
7. Kämpfer P, Arun A, Young CC, Chen WM, Sridhar KR, Rekha PD. *Chryseobacterium arthrosphaerae* sp. nov., isolated from the faeces of the pill millipede *Arthrosphaeramagna Attems*. Int J Syst Evol Microb. 2010; 60: 1765–9.
8. Liang CY, Yang CH, Lai CH, Huang YH, Lin JN. Genomic Features, Comparative Genomic Analysis, and Antimicrobial Susceptibility Patterns of *Chryseobacterium arthrosphaerae* Strain ED882-96 Isolated in Taiwan. Genes. 2019; 10: 309.
9. Im JHM, Kim D, Jin JM, Kim EY, Park YK, Kwon HY, et al. *Chryseobacterium arthrosphaerae* ventriculitis A case report. Medicine. 2020; 99(34): e21751.
10. Zhong HR, Wang PL, Chen YF. Construction of *clpV* deletion mutant of avian pathogenic *Escherichia coli* and the impact of *clpV* on expression of type fimbriae. Chinese Journal of Veterinary Science. 2021;41(6):1105-10.
11. Chen LW, Zhang MM, Zhang JN, Huang L, Zhao L, Xu X, et al. Synergism of sodA, sodB and KatG in *Aeromonas hydrophila* under antioxidative stress. Journal of Fisheries of China. 2021;45(1):136-46.
12. Medrano DCL, Vega GA, Ruiz BE, Abel M, Mayra C. Moonlight protein induce protection in a mouse model against Candida species. Microb Pathogenesis. 2018;3(124):21-9.
13. Han ZQ, Cui ZJ. Reversible methionine residue oxidation in signalling proteins and methionine sulfoxide reductases. Acta Biophys Sin. 2012;26(10): 861-79.
14. Gao HJ, Cheng GY, Wang YL, Ning J, Chen T, Li J, et al. Research progress of the mainly bacterial efflux pumps and related regulator. Chinese Journal of Animal and Veterinary Sciences. 2017;48(11):2023-33.
15. Xiong F, Mou YZ. Detection of Gene Mutation Locus and Analysis of Resistant Substrate in Multiple Resistances System of *Neisseria Gonorrhoeae*. Sichuan Medical Journal. 2014; 35(8): 952-4.
16. Zhang KH, Zuo LL, Xu X, Wang Y. Study on the mRNA expression of active efflux system gene adeFGH of multi-drug resistant *Acinetobacter baumannii*. China Modern Doctor. 2018;56(32):5-12.

17. Chin CS, Peluso P, Sedlazeck FJ, Nattestad M, Concepcion GT, Clum A, et al. Phased diploid genome assembly with single-molecule real-time sequencing. Nat Methods. 2016;13(12):1050.
18. Hunt M, Silva ND, Otto TD, Julian P, Jacqueline A, Simon R. Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biol. 2015;16(1):294.
19. Tarailo-Graovac M, Chen N. Using RepeatMasker to identify repetitive elements in genomic sequences. Curr Protoc Bioinform. 2009;Chapter 4:1-14.
20. Akhter S, Aziz RK, Edwards RA. PhiSpy: a novel algorithm for finding prophages in bacterial genomes that combines similarity-and composition-based strategies. Nucleic Acids Res. 2012;40(16):e126.
21. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 1997;25(5):955-64.
22. Lagesen K, Hallin P, Rødland EA, Staerfeldt H, Rognes T, Ussery H. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 2007;35(9): 3100-8.
23. Griffiths-Jones S, Bateman A, Marshall M, Khanna A, Eddy SR. Rfam: an RNA family database. Nucleic Acids Res. 2003;31(1):439-41.
24. Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, et al. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res. 2011; 39: W316–22.
25. Cantarel BL, Coutinho PM, Rancurel C, Thomas B, Vincent L, Bernard H. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res. 2008;37(suppl_1): D233-8.
26. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods. 2015;12:59-60.
27. Sean R. A new generation of homology search tools based on probabilistic inference. Genome Inform. 2009;23:205-11.
28. Winnenburg R, Baldwin TK, Urban M, Rawlings C, Köhler J, Hammond-Kosack KE. PHI-base: a new database for pathogen host interactions. Nucleic Acids Res. 2006; 34(suppl_1): D459-64.
29. Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, et al. VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res. 2005;33(suppl_1):D325-8.
30. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, et al. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 2017;45(D1):D566-73.
31. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19(9):1639-45.
32. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. CLSI: Wayne, PA, USA, 2020.
33. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-25.

34. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870-4.
35. Lee I, Kim YO, Park SC, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microb. 2015;66:1100–3.
36. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA. 2009;106:19126–31.

Figures

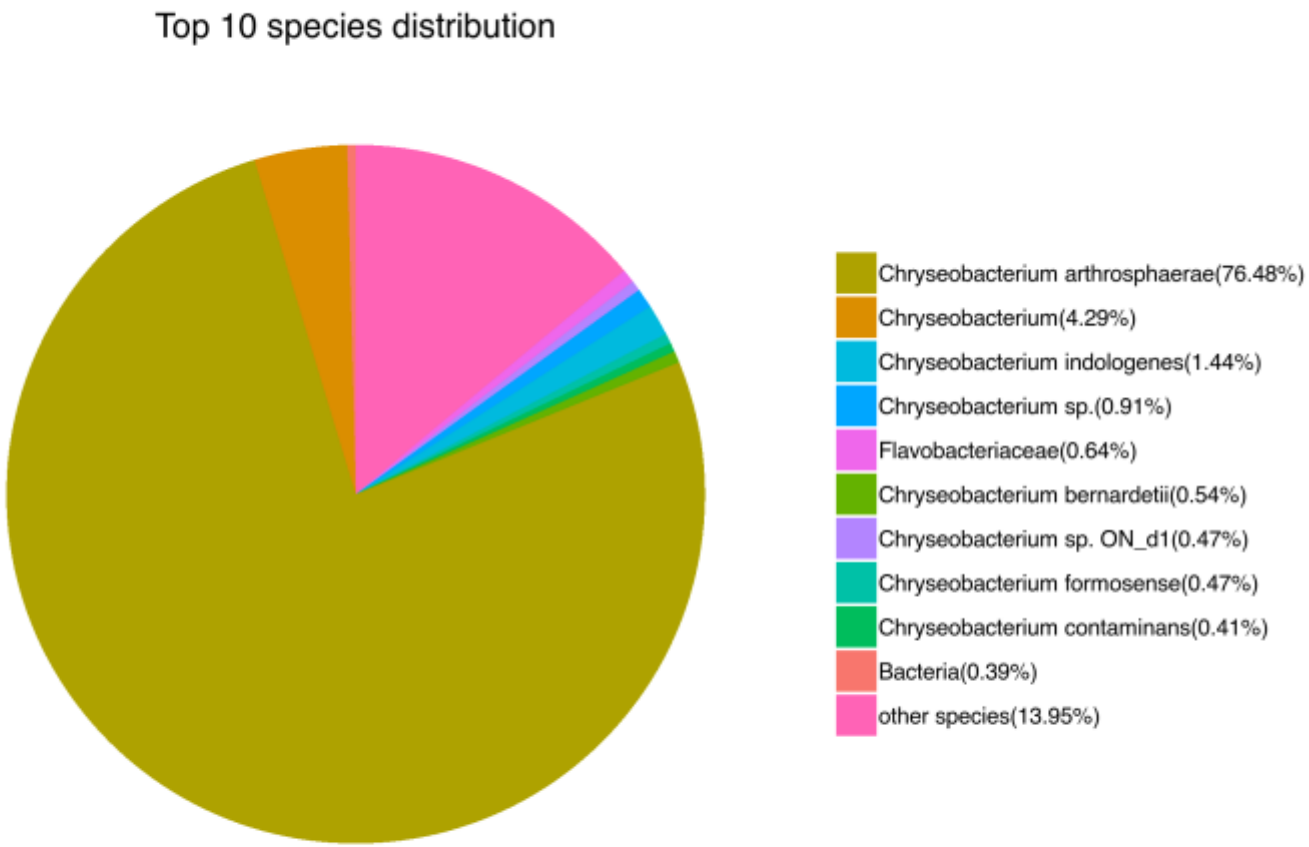

Figure 1

NR analysis result of strain FS91703. Different colored sectors indicate the percentage of each species.

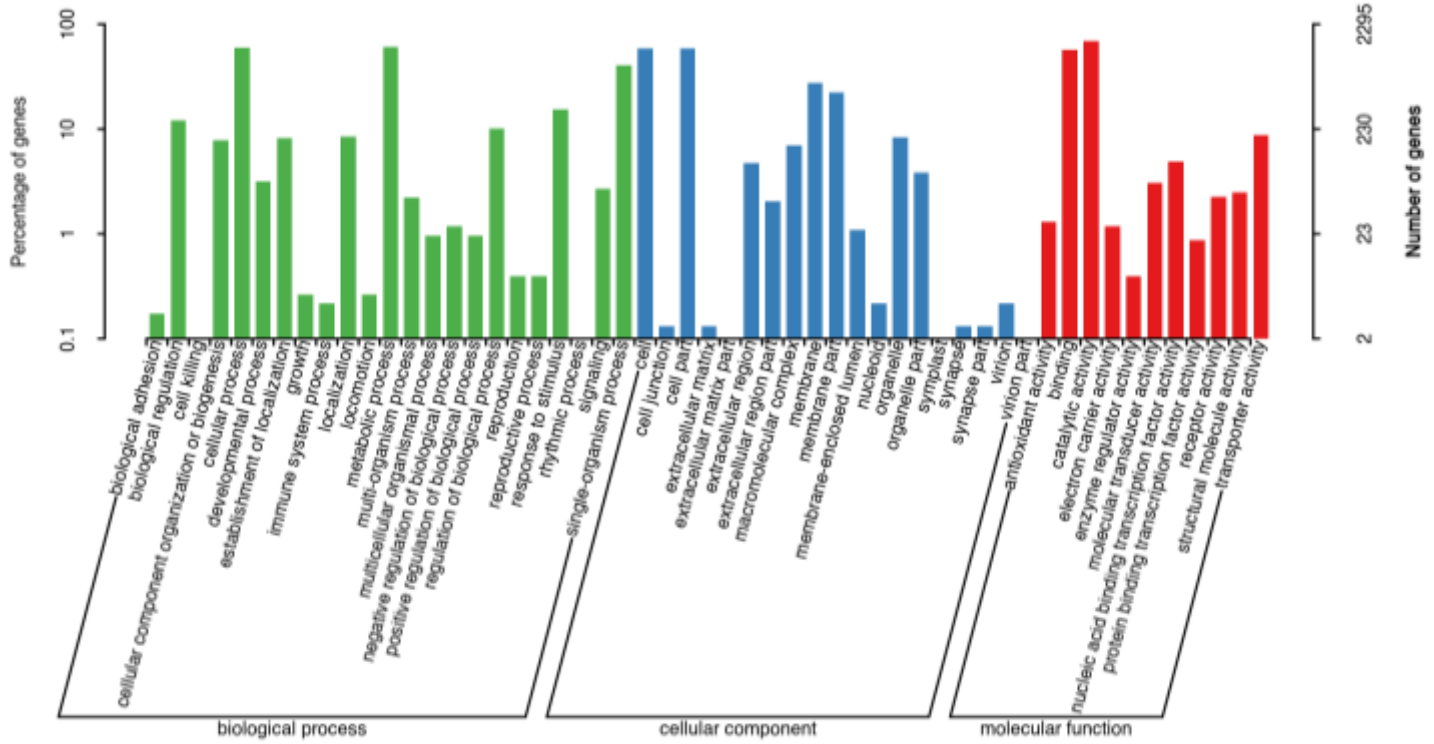

Figure 2

GO analysis for the result of strain FS91703

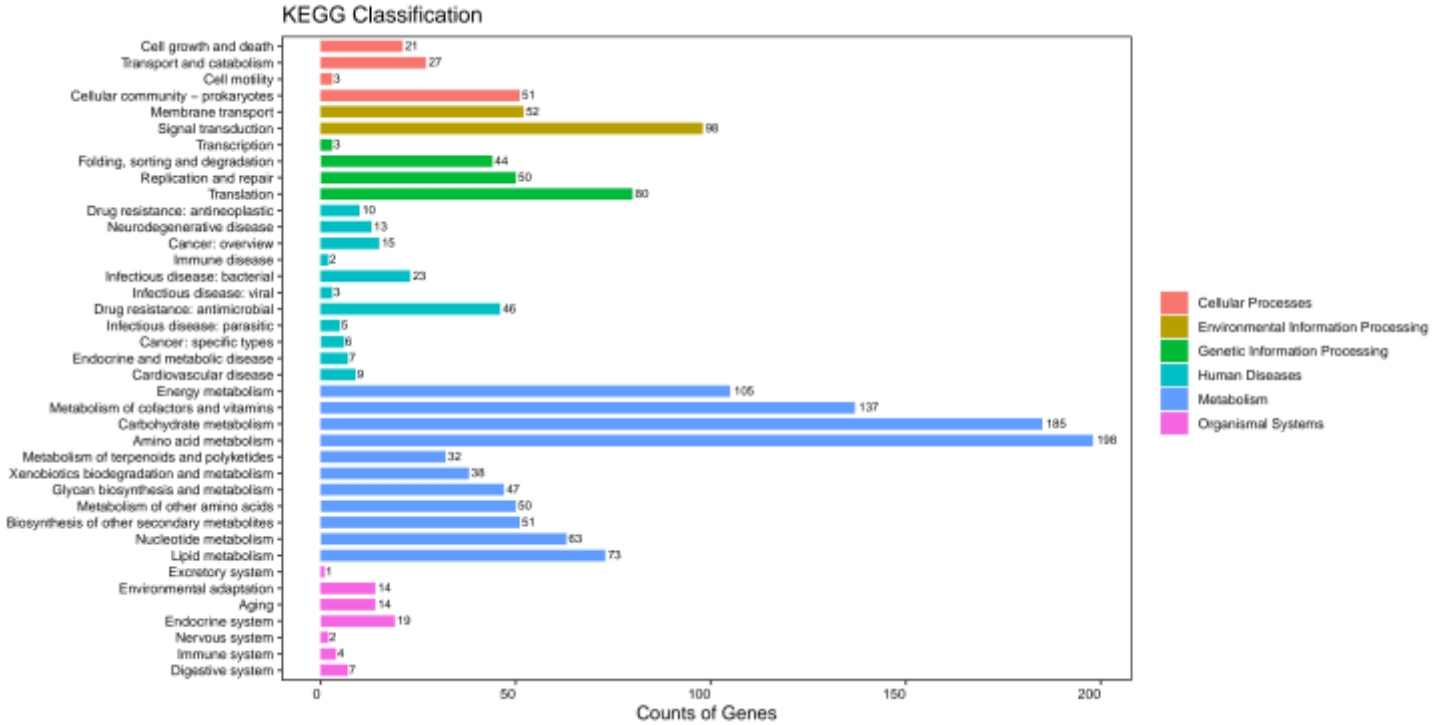

Figure 3

KEGG analysis for the result of strain FS91703

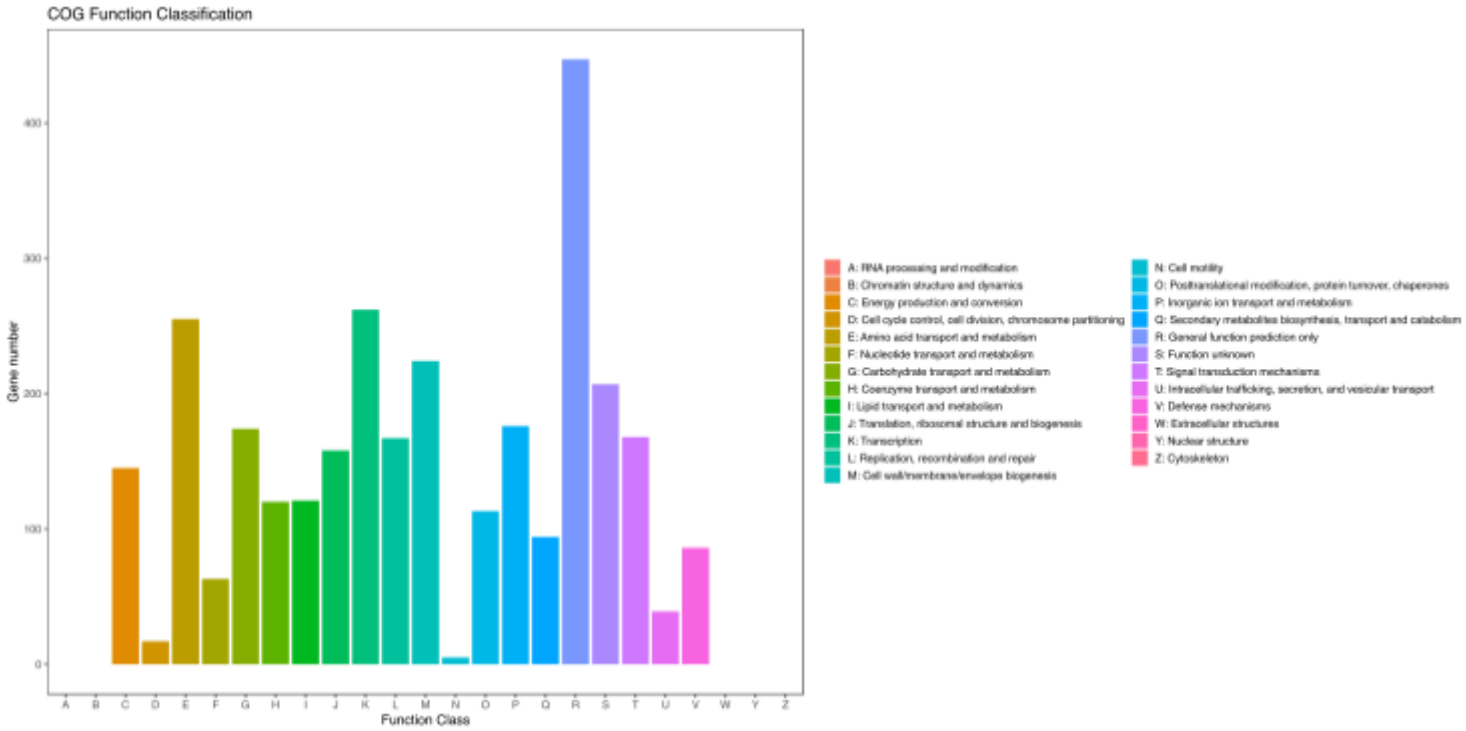

Figure 4

COG analysis for the result of strain FS91703

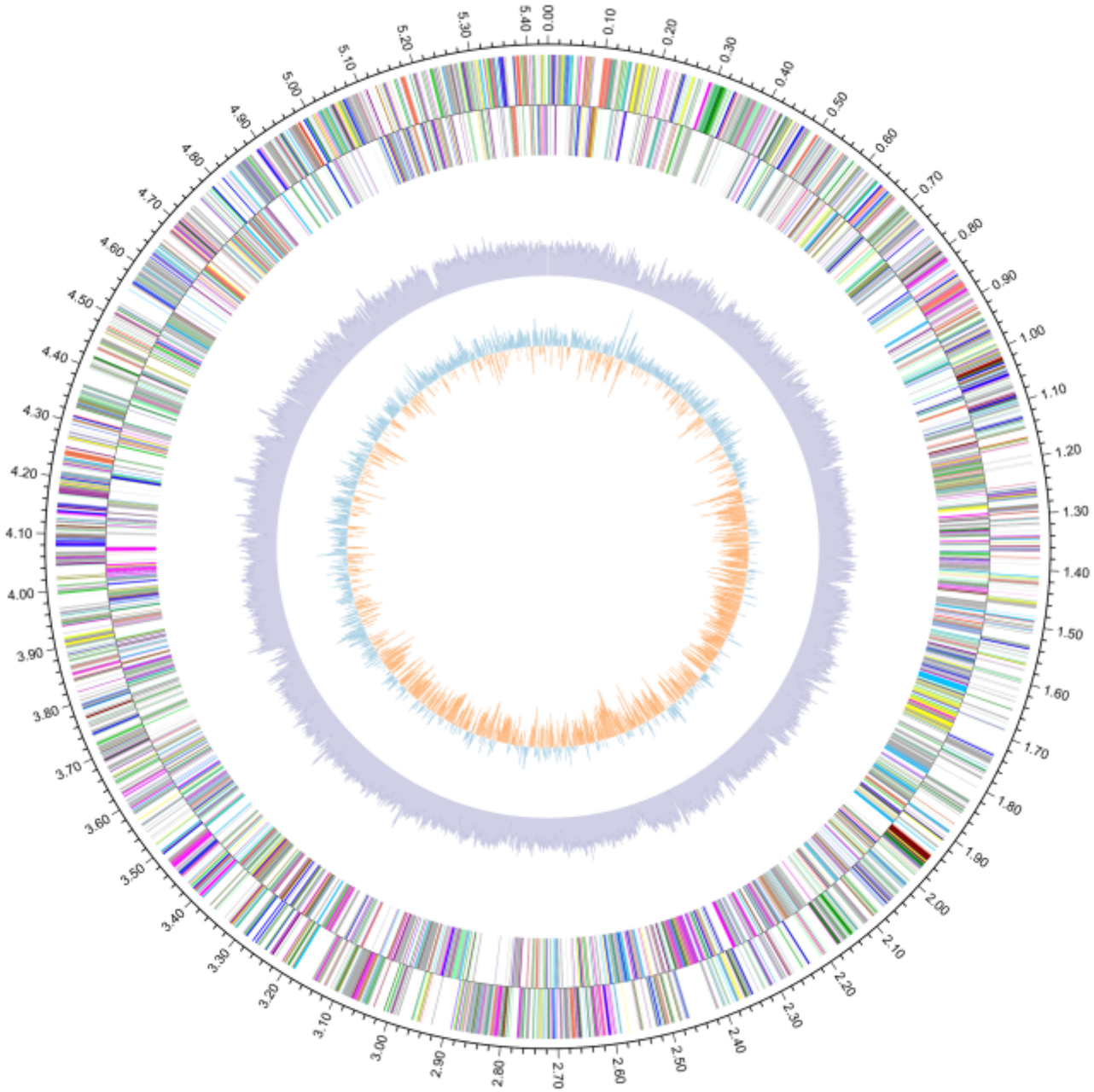

Figure 5

Genome-wide map of Strain FS91703. Coding region prediction with color-coded by role category was indicated by circles. COG annotation, COG annotation for lagging strand, non-coding RNA (green, sRNA; blue, tRNA; red, rRNA), GC content, and GC skew for leading strand are listed from outside to inside.

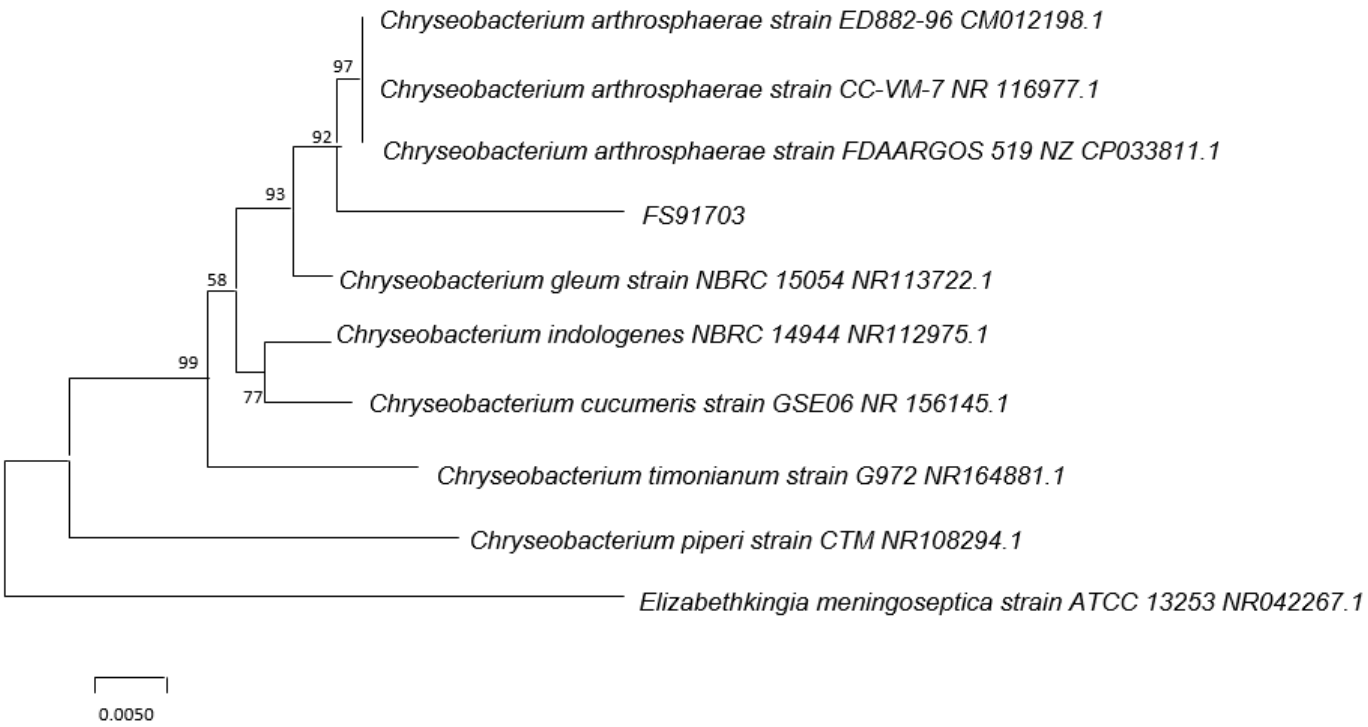

Figure 6

Phylogenetic tree with 16S rRNA sequencing of strain FS91703

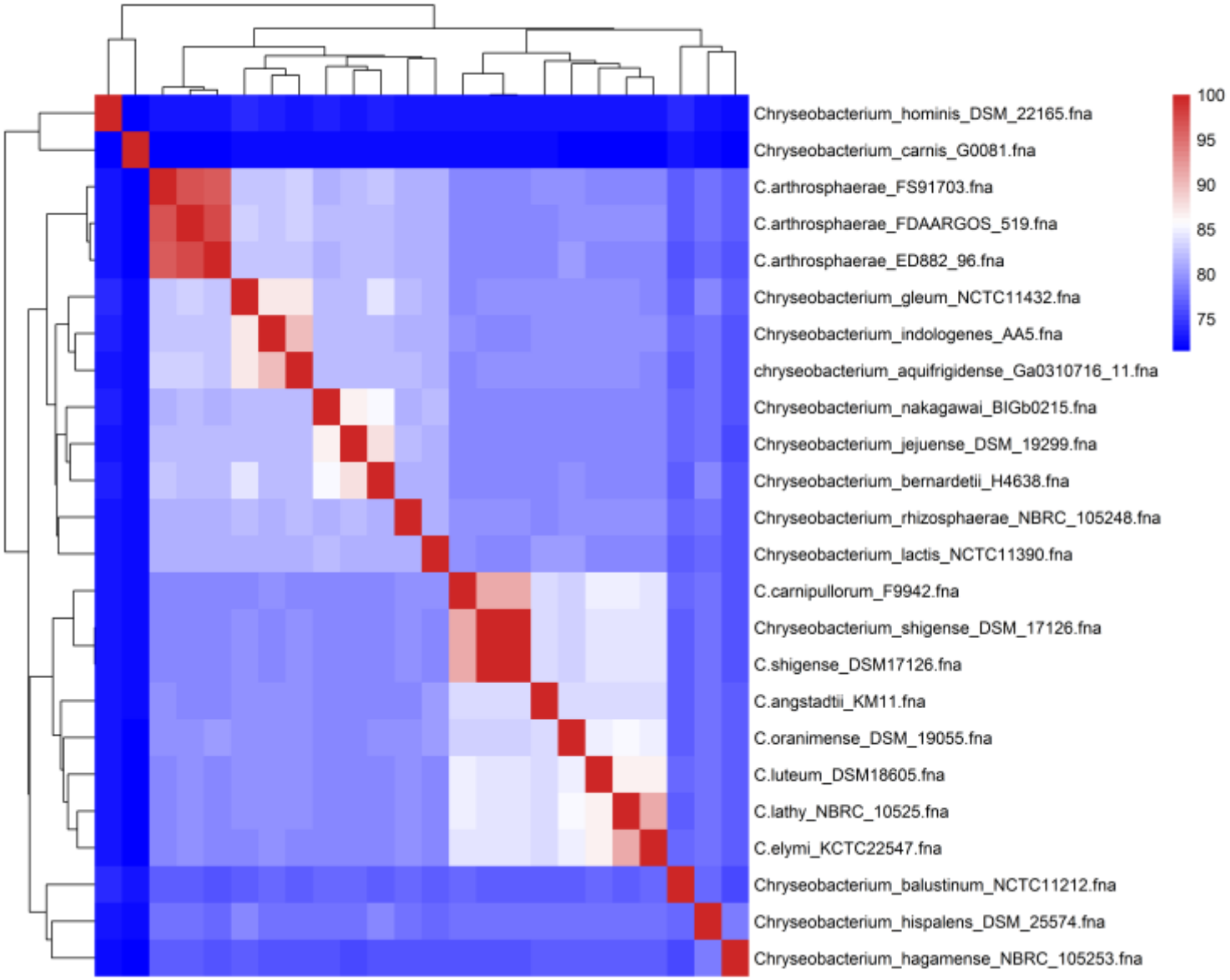

Figure 7

A heat map of the average nucleotide sequence identity (ANI) between strain FS91703 and 23 *Chryseobacterium* species