Progress in Microbes and Molecular Biology

HH PUBLISHER

Original Research Article

Genomic and Resistome Analyses of *Elizabethkingia anophelis* Strain B2D isolated from Dental Plaque of Patient

Share Yuan Goh[1], Kah-Ooi Chua[1,2*], Saad Ahmed Khan[3], Noor Hayaty Abu Kasim[3], Yvonne Jing Mei Liew[4,5], Yin Yin Lau[6], Kar-Wai Hong[7], Wai-Fong Yin[1], Hoi-Sen Yong[1], Chien-Yi Chang[8], Kok-Gan Chan [1,7,9,10*]

Article History

Received: 13 February 2024;

Received in Revised Form: 01 April 2024;

Accepted: 12 April 2024;

Available Online: 17 April 2024

[1]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia; yuanny_goh@hotmail.com (SYG); yinwaifong@yahoo.com (WYF); yong@um.edu.my (HSY)

[2]Centre for Research in Biotechnology for Agriculture (CEBAR), Universiti Malaya, Kuala Lumpur 50603, Malaysia

[3]Department of Restorative Dentistry, Faculty of Dentistry, Universiti Malaya, 50603, Kuala Lumpur, Malaysia; saadkhanz@gmail.com (SAK); nhayaty@um.edu.my (NHAK)

[4]Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, Universiti Malaya, 50603 Kuala Lumpur, Malaysia; yvonneljm@um.edu.my (YJML)

[5]University of Malaya Centre for Proteomics Research, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

[6] Department of Biotechnology, Faculty of Applied Sciences, UCSI University, Kuala Lumpur, Malaysia; LauYY@ucsiuniversity.edu.my (YYL)

[7]Novel Bacteria and Drug Discovery Research Group (NBDD), Microbiome and Bioresource Research Strength (MBRS), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway 47500, Malaysia; sebastian_karwai@yahoo.com (KWH)

[8]School of Dental Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom; chienyi.chang@newcastle.ac.uk (CYC)

[9]Institute of Marine Sciences, Shantou University, Shantou, China

[10]Microbiome Research Group, Research Center for Life Science and Healthcare, Nottingham Ningbo China Beacons of Excellence Research and Innovation Institute (CBI), University of Nottingham Ningbo China (UNNC), Ningbo 315000, China

*Corresponding authors: Kok-Gan Chan and Kah-Ooi Chua; Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia; kokgan@um.edu.my (K-GC); kahooi@um.edu.my (K-OC)

Abstract: In this study, strain B2D isolated from a dental plaque sample of a human patient was studied for its general characteristics, taxonomic identification, genome features, and resistome profile. The bacterium exhibited antibiotic resistance to all beta-lactam antibiotics, nitrofuran, and sulfonamides, with high minimum inhibitory concentrations. It was only sensitive to the fluoroquinolone ciprofloxacin and intermediately susceptible to aminoglycoside tobramycin. A preliminary identification through 16S rRNA gene sequences revealed that it shared the highest sequence identity with *Elizabethkingia anophelis* subsp. *endophytica* JM-87^T (100%) and *Elizabethkingia anophelis* subsp. *anophelis* R26^T (99.31%). The draft genome of strain B2D was approximately 3.9 Mbp with 50 contigs and 35.5% GC content. A 16S rRNA gene and core genes-based phylogenetic analyses revealed a close phylogenetic relationship between strain B2D and the other *Elizabethkingia* type strains. An above species level threshold average nucleotide identity value confirmed its taxonomic identity as *Elizabethkingia anophelis*. Furthermore, we conducted a resistome analysis of strain B2D and *Elizabethkingia* type strains, revealing the presence of widespread antibiotic resistance genes, including beta-lactamases and genes associated with cationic antiseptic resistance and glycopeptide resistance. Overall, the multidrug resistant profile of strain B2D as elucidated and confirmed through whole genome analysis indicated its potential as a reservoir of beta-lactamase genes. Moreover, its presence within dental plaque in the human oral cavity prompts speculation regarding its role as an opportunistic pathogen capable of causing infections, particularly in immunocompromised individuals.

Keywords: VITEK 2, beta-lactamase, opportunistic pathogen, Weeksellaceae, antibiotic resistance; SDG 3 Good health and well-being

1. Introduction

The bacterial genus *Elizabethkingia* within the family Weeksellaceae is comprised of Gram-negative, rod-shaped, and aerobic species. Members of this genus are commonly found in the environments but they have been isolated from different plants and animals, including humans [1]. There are seven species in the genus *Elizabethkingia*: *E. anophelis*, *E. meningoseptica*, *E. miricola*, *E. occulta*, *E. bruuniana*, *E. ursingii* and *E. argenteiflava*. The type strains of these species were isolated from diverse environments including plants [2, 3], environmental samples such as soil [4] and condensation water in space station [5], insect midgut [6] as well as clinical samples such as cerebrospinal fluid of premature infant [7], human blood, and sputum [4].

Elizabethkingia anophelis was first isolated from the midgut of the mosquito *Anopheles gambiae* [6]. It has been reported as a plant endophyte [3], human pathogen [8], environment bacterium in industrial wastewater samples [9], and various aquatic environments [1]. Currently, there are two subspecies under *E. anophelis*, *E. a*. subsp. *endophytica* and *E. a*. subsp. *anophelis*[3]. *E. anophelis* strains displayed broad antibiotic resistance, notably to broad-spectrum beta-lactams, aminoglycosides, tetracyclines, and varying levels of resistance to macrolides and fluoroquinolones [9, 10]. Together with *E. meningoseptica* and *E. miricola*, *E. anophelis* is one of the three medically important species in the genus *Elizabethkingia* [9]. The high genetic relatedness between *E. anophelis* and *E. meningoseptica* often leads to misidentifications in clinical settings [11]. To address these challenges, molecular methods, including 16S rRNA gene sequencing and whole genome sequence-based analyses, have been employed to provide accurate taxonomic identification.

In this study, strain B2D of *E. anophelis* that was isolated from the dental plaque of a patient was assessed for its antibiotic susceptibility, and its genome was sequenced using Illumina technology. Through whole genome analysis, a comprehensive investigation was performed to elucidate its genomic characteristics and antibiotic resistance profile. The whole genome sequences of strain B2D and closely related *Elizabethkingia* species were included for a phylogenomic analysis and calculation of overall genome-relatedness to provide a precise taxonomic classification. This study then further investigated the resistome of strain B2D and all type strains of *Elizabethkingia* species. These findings will serve as the foundation for a deeper understanding of the genome characteristics and resistome of *Elizabethkingia* species.

2. Methods

2.1. Dental Plaque Sample Collection and Processing

This study was conducted with medical ethics approval from the Faculty of Dentistry (Universiti Malaya), Ethics and Research Committee (DFRD-1302/0033-L). Briefly, dental plaque samples were collected from the patient before tooth extraction. The buccal or proximal surfaces of carious tooth subgingival pockets were carefully picked for soft and loose dental plaque using sterile Gracey curettes. The collected samples were kept in sterile Sorensen phosphate-buffered saline (PBS, pH 7.6) in a specimen bottle and transported to the laboratory for immediate processing. In the laboratory, the samples in PBS were homogenized by a vortexer, and 100 µL of the saline sample was cultured on Columbia Agar (Isolac, Isolab, Malaysia) supplemented with 5% (v/v) sheep blood and aerobically cultured for 24 hr at 37˚C. Pure cultures of all isolates were obtained on sheep blood agar.

2.2. Isolation and Identification of Strain B2D

All isolates were characterized using biochemical tests including Gram staining, catalase, and oxidase tests. Strain B2D was selected for further study and was routinely maintained on tryptic soy medium and stored long-term in 20% (v/v) glycerol stock in an ultralow freezer under -80 °C.

The strain was molecularly identified by 16S rRNA gene amplification and analysis. Briefly, the 16S rRNA gene of strain B2D was amplified using the primer pair 27F: 5′–AGA GTT TGA TCM TGG CTC AG–3′ and 1492R: 5′–AAG GAG GTG WTC CAR CC–3′ [12] and the gene amplicons were subjected to commercial Sanger sequencing (Apical Scientific Sdn. Bhd., Selangor, Malaysia). The DNA sequences were analyzed using Molecular Evolutionary Genetic Analysis (MEGA) version 11.0 [13]. The sequences were quality-filtered for ambiguous nucleotides, and assembled into final 16S rRNA gene sequences. The preliminary identification of the strain was determined through a BLAST search with reference to the EzBioCloud server (http://www.ezbiocloud.net/eztaxon) [14, 15] and the NCBI GenBank database [16]. The same sequence was mapped against the expanded Human Oral Microbiome Database (HOMD) for 16S rRNA sequence identification [17].

2.3. Antibiotic Susceptibility Testing with VITEK 2 system

Strain B2D was cultured on tryptic soy agar (TSA) and incubated at 37°C for 18 hr. Single colonies of the culture were suspended in 0.45% saline and adjusted to an absorbance value of 0.08 at 600 nm, which was equivalent to 0.5 MacFarland standard using a spectrophotometer. The adjusted suspension was used as inoculum for the test card AST-GN66 of the VITEK 2 system. The antimicrobials contained in the AST-GN66 test card are listed in Table 1. With the results from the VITEK 2 system, the minimal inhibitory concentration (MIC) and the susceptibility of strain B2D to each antibiotic were determined and interpreted by the Advanced Expert System (AES).

2.4. Genomic DNA Extraction and Sample Preparation for Whole Genome Sequencing

The genomic DNA of strain B2D was extracted using a MasterPure Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA) [18]. The extracted DNA was eluted in EB buffer (Qiagen). The quality and quantity of DNA were examined using a NanoDrop spectrophotometer (Thermo Scientific, USA) [19] and Qubit 2.0 fluorometer (dsDNA High Sensitivity Assay Kit) (Invitrogen, USA), respectively [20]. Purified genomic DNA of strain B2D was stored at -20 °C and used for sample preparation for whole genome sequencing.

The DNA library for sequencing was prepared with the Illumina Nextera DNA sample preparation kit (Illumina, Inc., CA) [21-23]. The quality control of the DNA library template was done on Bioanalyzer while its concentration was determined by qPCR as instructed in the KAPA Library Quantification Kits for Illumina sequencing platforms (KAPA BioSystems, Boston, MA, USA) and diluted to 2 nM in Tris-HCl (10 mM, pH 8.5) with 0.1% (v/v) Tween 20. The library was denatured and further diluted to 10 pM before loading into the cartridge for high throughput sequencing with the Illumina MiSeq personal sequencer platform (Illumina, Inc., CA) [24].

2.5. Genome Assembly, Annotation and Analysis

The generated raw reads with a quality lower than Q20 were filtered, all the ambiguous nucleotides were trimmed and *de novo* assembled with CLC Genomic Workbench (version 7.0.4) [25]. Contigs with a minimal length of 200 bp were selected for subsequent analysis. The quality of the assembled genome was assessed using QUAST v5.2 [26], while the genome completeness was assessed using BUSCO v5.4.6 with reference to the Flavobacteriales lineage [27], respectively.

The genes in the genome of strain B2D were annotated with RAST (Rapid Annotation using Subsystem Technology version 4) [28] and Prokaryotic Genome Annotation Pipeline (PGAP) [29, 30]. The 16S rRNA gene sequence identified in PGAP was extracted for a phylogenetic analysis. Web-based annotation tools eggNOG-mapper (v.2.1.6) (http://eggnog-mapper.embl.de/) was utilized for additional annotation of the putative function of the genes based on orthologs [31]. Genes associated with antibiotic resistance were detected using the Resistance Gene Identifier (RGI) of the Comprehensive Antibiotic Resistance Database (CARD) with the parameters “Perfect and strict hits only” and “High quality/coverage” [32]. The most closely related neighbour taxa were identified from the EzBioCloud database and the gene data were obtained from GenBank for a phylogenetic analysis in MEGA version 11 [13] using the maximum likelihood algorithm and bootstrap analysis of 1000 replicates. A neighbour taxon from the same family, *Epilithonimonas tenax* DSM 16811^T which was isolated from epilithon, was included in the analysis as the outgroup taxon.

2.6. Phylogenomic Analysis and Calculation of Average Nucleotide Identity (ANI)

The whole genome sequences of all *Elizabethkingia* type strains were downloaded from the GenBank for phylogenomic analysis and only genomes with >96% BUSCO genome completeness were included. *Epilithonimonas tenax* DSM 16811^T, a neighbour taxon from the same family was included in the analysis as the outgroup taxon. The taxonomic identity

of the strain B2D was further confirmed through the calculation of average nucleotide identity (ANI) through the Orthologous Average Nucleotide Identity Tool (OAT) [33]. Pan-genome analysis was performed using Roary 3.13.0 [34] at 85% minimum percentage identity for BLASTp to identify the core genes and an alignment of the core genes was produced. Subsequently, the core gene alignment was used for a maximum likelihood phylogenomic analysis using IQ-TREE 2.2.0 [35], with 1000 times bootstrap replications. The resulting phylogenomic tree was visualized using MEGA 11[13].

3. Results

3.1. Biochemical and Molecular Identification of Strain B2D

Strain B2D was determined as a Gram-negative, rod-shaped, catalase, and oxidase negative bacterium (Table 1). The bacterium grew optimally at 37 °C and appeared as creamy white colonies on Columbia agar supplemented with 5% sheep blood, with no hemolytic activity observed. A preliminary identification by 16S rRNA gene analysis revealed that the strain shared 100% sequence identity with E. anophelis subsp. endophytica JM-87^T, and 99.31% sequence identity with E. anophelis subsp. anophelis R26^T, indicating a close phylogenetic relationship between strain B2D and E. anophelis.

3.2. Antibiotic Susceptibility of Strain B2D

The antibiotic susceptibility of strain B2D was tested using VITEK 2 (bioMérieux, Inc., Marcy l'Etoile, France), an automated commercial antimicrobial susceptibility test system that utilizes a new fluorescence-based technology to study individual biochemical reactions run in parallel which are contained in a variety of microbe identification cards. Following inoculation with a standardized microbiological sample to be screened, each card is incubated and scanned by the VITEK 2 instrument. The results generated were compared with the internal VITEK 2 database of known species-specific reactions [36].

Based on VITEK 2 analysis, strain B2D showed high level of resistance towards all beta-lactam antibiotics in the card tested including penicillin (MIC ≥ 32 μg/mL), the combination of penicillin and beta-lactamase inhibitors (MIC ≥ 32 μg/mL), carbapenem (MIC ≥ 16 μg/mL), first to fourth generation cephalosporins (MIC ≥ 64 μg/mL), nitrofuran (MIC = 80 μg/mL), sulfonamides (MIC ≥ 16 μg/mL) as well as the aminoglycoside gentamicin (MIC ≥ 16 μg/mL) and levofloxacin which is a fluoroquinolone (MIC ≥ 512 μg/mL). The strain only exhibited intermediate susceptibility to aminoglycoside tobramycin (MIC = 2 μg/mL) and was sensitive to the fluoroquinolone ciprofloxacin (MIC = 0.5 μg/mL) (Table 2).

Table 1. General features and genome information of strain B2D.

General features	Description
Classification	Domain Bacteria
	Phylum Bacteroidota
	Class Flavobacteriia
	Order Flavobacteriales
	Family Weeksellaceae
	Genus *Elizabethkingia*
	Species *anophelis*
	Strain B2D
Gram staining	Negative
Cell shape	Rod
Pigmentation	Creamy white on blood agar
Optimal growth temperature	37 °C
Genome information	
BioProject accession	PRJNA248328
BioSample accession	SAMN02797821
GenBank accession	JNCG00000000
Sequencing technology	Illumina MiSeq
Genome coverage	121.8×
Assembly method	CLC Genomics Workbench v. 7
Genome attribute	
Genome size (bp)	3,936,249
Number of contig	50
GC content (%)	35.5
Total genes	3,552
Coding DNA sequences (CDS)	3,466
Pseudo Genes	42
rRNAs	3 (5S, 16S, 23S)
tRNAs	40

Table 2. Antibiotic susceptibility of strain B2D tested using VITEK 2 test card AST-GN66 in a VITEK 2 system as interpreted by AES (R: resistant; I: intermediate; S: sensitive).

Antibiotic class	Antimicrobials	MIC (μg/ml)	AES Interpretation
Penicillin	Ampicillin	≥ 32	R
Penicillin/β-lactamase inhibitor	Ampicillin/Sulbactam	≥ 32	R
	Piperacillin/Tazobactam	≥ 128	R
1st gen cephalosporin	Cefazolin	≥ 64	R
2nd gen cephalosporin	Cefoxitin	≥ 64	R
3rd gen cephalosporin	Ceftazidime	≥ 64	R
	Ceftriaxone	≥ 64	R
4th gen cephalosporin	Cefepime	≥ 64	R
Carbapenem	Ertapenem	≥ 16	R
	Imipenem	≥ 16	R
Aminoglycoside	Gentamicin	≥ 16	R
	Tobramycin	2	I
Fluoroquinolone	Ciprofloxacin	0.5	S
	Levofloxacin	≥512	R
Nitrofuran	Nitrofurantoin	80	R
Sulfonamides	Trimethoprim/ Sulfamethoxazole	≥ 16	R

3.3. Genome Features of Strain B2D

The assembled genome of strain B2D comprised 3,936,249 bp distributed across 50 contigs, with a GC content of 35.5%. There was a total of 3,552 genes, of which 3,466 were CDS, 3 were rRNAs and 40 were tRNAs (Table 1). The draft genome scored a completeness of 97.1% in BUSCO analysis (Table 3). This Whole Genome Shotgun project is deposited at DDBJ/EMBL/GenBank under the accession JNCG00000000. The version described in this paper is the first version, JNCG01000000.

Functional annotation of the genes using eggnog-mapper revealed that 26% of the genes were not known for their functions. Other than that, the majority of the genes were involved in basic cellular processes such as transcription (COG category K, 9%), amino acid transport and metabolism (E, 8%), and cell wall/membrane/envelope biogenesis (M, 8%). Notably, approximately 2% of the genes were assigned to category V, which was responsible for the defence mechanism (Figure 1).

Table 3. List of *Elizabethkingia* taxa and genomes included for analysis in this study.

Taxon	Strain name	16S rRNA gene accession number	16S rRNA gene sequence similarity with B2D	GenBank accession number	Genome length (bp)	Contig number	BUSCO completeness (%)	Origin	Reference
Elizabethkingia anophelis	B2D	JNCG01000000	-	JNCG01000000	3,936,249	50	97.1	Human dental plaque	This study
Elizabethkingia anophelis subsp. *endophytica*	JM-87(T)	KP113704	100	CP016372	4,184,507	1	96.7	Plant tissue	[3]
Elizabethkingia anophelis subsp. *anophelis*	R26(T)	CP023401	99.31	CP023401	4,058,311	1	97.0	Midgut of mosquito Anopheles gambiae	[6]
Elizabethkingia miricola	DSM 14571(T)	jgi.1048980	97.92	VNHK00000000	4,294,661	38	97.2	Condensation water in space station	[7]
Elizabethkingia meningoseptica	ATCC 13253(T)	ASAN01000081	97.85	ASAN00000000	3,796,928	115	96.0	Cerebrospinal fluid of premature infant	[7]
Elizabethkingia bruuniana	G0146(T)	CP014337	97.85	CP014337	4,433,522	1	97.3	Human blood	[4]

Taxon	**Strain name**	**16S rRNA gene accession number**	**16S rRNA gene sequence similarity with B2D**	**GenBank accession number**	**Genome length (bp)**	**Contig number**	**BUSCO complete-ness (%)**	**Origin**	**Reference**
Elizabethkingia occulta	G4070(T)	MAHX01000009	97.78	MAHX00000000	4,154,012	21	97.2	Human sputum	[4]
Elizabethkingia ursingii	G4122(T)	LNOK01000028	97.57	LNOK00000000	4,339,237	53	97.0	Soil	[4]
Elizabethkingia argenteiflava	YB22(T)	KY510834	95.81	JAAABJ000000000	2,702,182	689	96.3	Soybean pod	[2]
Epilithonimonas tenax	DSM 16811(T)	AUAA01000078	94.3	AUAA00000000	3,624,509	109	99.9	Epilithon-covered stones	[37]

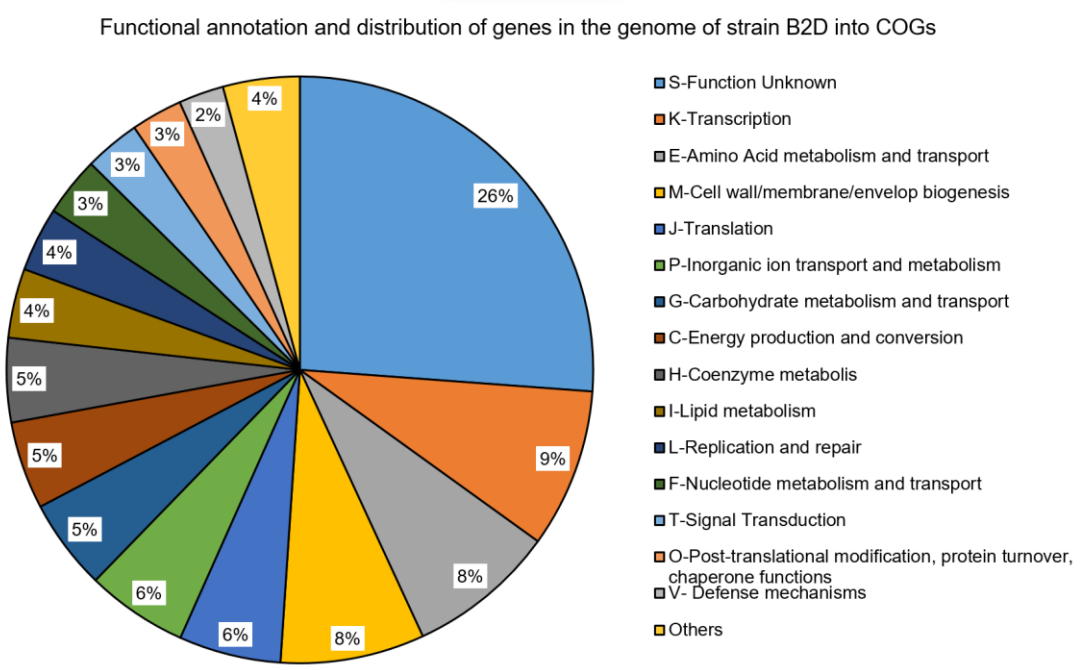

Figure 1. Functional annotation and distribution of genes in the genome of strain B2D into COG categories.

3.4. Phylogenomic Analysis and Taxonomic Placement of Strain B2D

A maximum likelihood phylogenetic analysis constructed based on the 16S rRNA gene extracted from the whole genome sequences showed that strain B2D formed a sister group with *E. anophelis* subsp. *endophytica* JM-87^T, in the same lineage as *E. anophelis* subsp. *anophelis* R26^T, with 100% bootstrap values. However, the support for some *Elizabethkingia* species was relatively low (Figure 2).

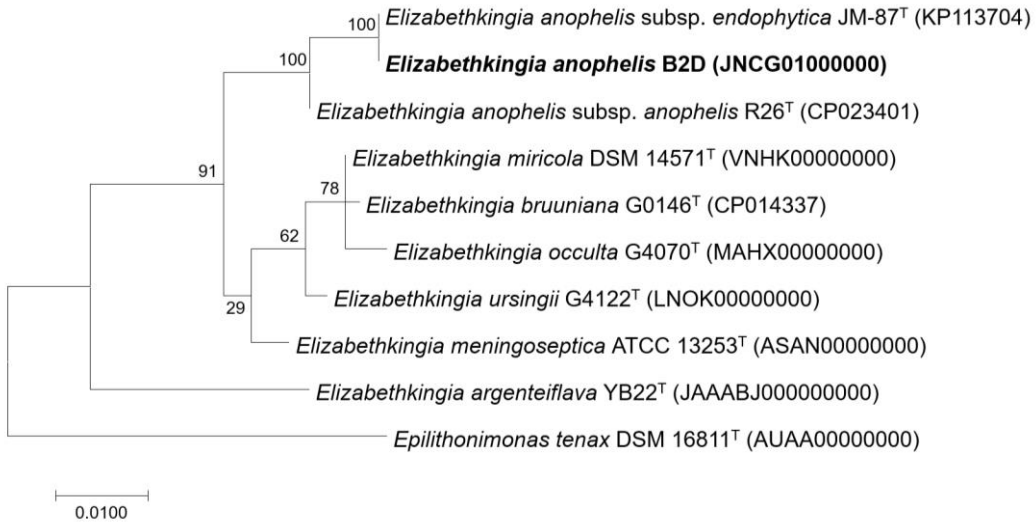

Figure 2. Maximum likelihood phylogenetic tree constructed using 16S rRNA genes of strain B2D and type strains of *Elizabethkingia*. *Epilithonimonas tenax* DSM 16811^T served as the outgroup taxon. Numbers displayed at nodes are bootstrap values, based on 1000 replications.

A total of 187 core genes were identified using Roary from the genomic data of nine *Elizabethkingia* strains and the outgroup taxon *Epilithonimonas tenax* DSM 16811^{T}. Functional annotation of these core genes using eggNOG-mapper classified them into various functional categories and indicated their potential biological roles across the related species. Notably, translation, ribosomal structure, and biogenesis (COG category J) was the most represented category with 59 out of 187 core genes, followed by 20 core genes in the energy production and conversion (C) category, and 17 core genes in the nucleotide transport and metabolism (H) (Figure 3).

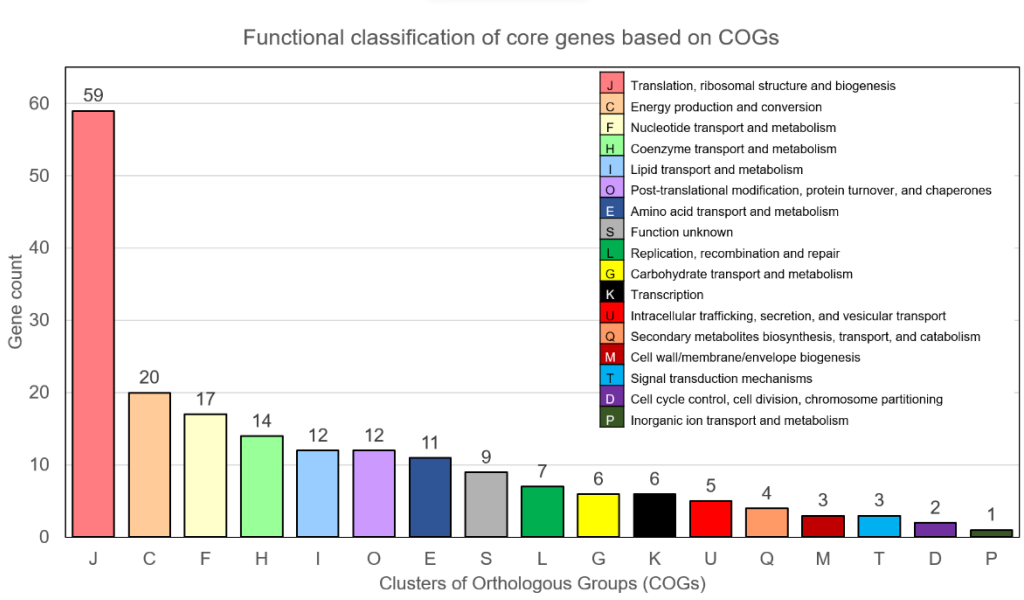

Figure 3. Functional classification of 187 core genes identified by Roary from the genomes of strain B2D, *Elizabethkingia* type strains and the outgroup taxon *Epilithonimonas tenax* DSM 16811^{T}.

The phylogenomic analysis, utilizing the DNA sequences of the 187 core genes (Figure 4), displayed a topology closely resembling the 16S rRNA gene-based phylogenetic tree (Figure 2). Strain B2D formed a sister group with *E. anophelis* subsp. *endophytica* JM-87^{T} in the same lineage as *E. anophelis* subsp. *anophelis* R26^{T}. The majority of the nodes exhibited 100% bootstrap support, indicating a more robust placement of strain B2D within the phylogeny. The placement for the strain *Elizabethkingia meningoseptica* ATCC 13253^{T} (Figure 4) however differed from that of the 16S rRNA gene phylogenetic tree (Figure 2).

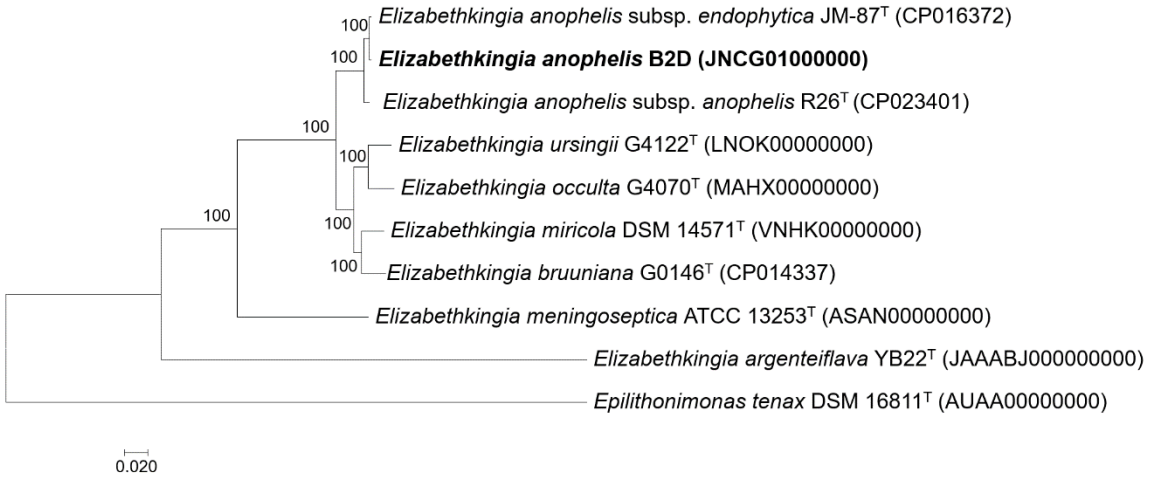

Figure 4. Maximum likelihood phylogenetic tree constructed using 187 core genes of strain B2D and type strains of *Elizabethkingia*. *Epilithonimonas tenax* DSM 16811^T served as the outgroup taxon. Numbers displayed at nodes are bootstrap values, based on 1000 replications.

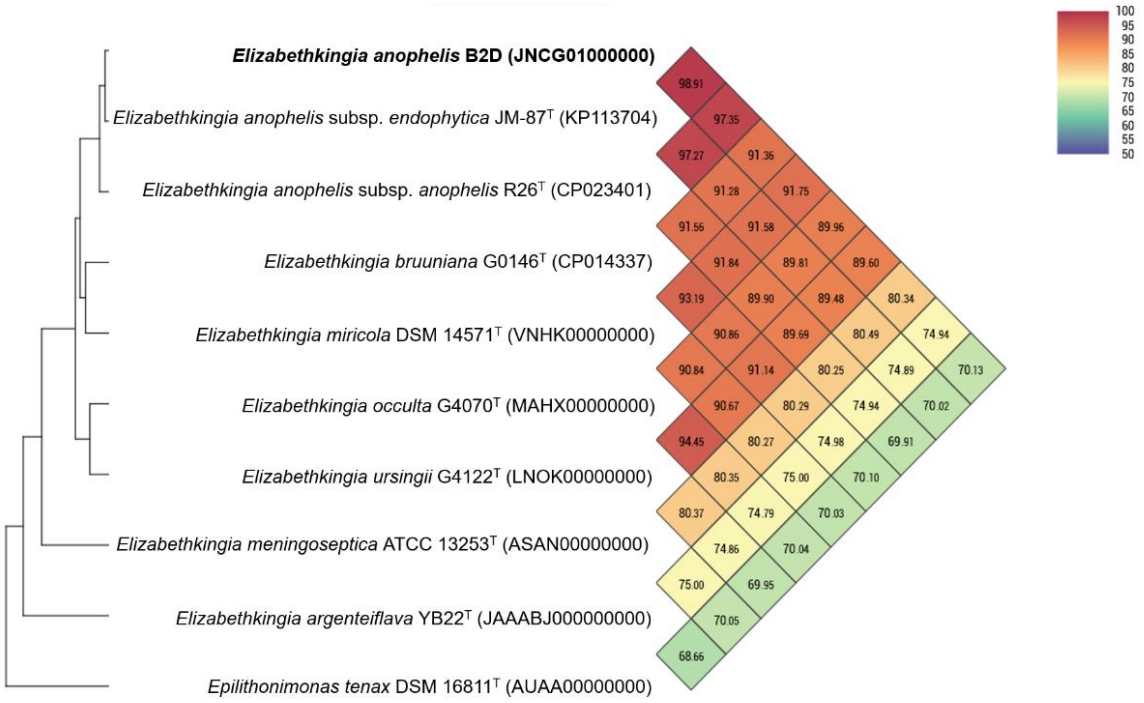

Figure 5. Average nucleotide identity (ANI) values between strain B2D, various *Elizabethkingia* type strains and the outgroup taxon *Epilithonimonas tenax* DSM 16811^T. ANI values above 95% were denoted by red colour, signifying high genomic coherence and indicative of strains belonging to the same species. Colour intensity fades as ANI values decrease and fall below the 95% threshold, suggesting genomic dissimilarity and divergence between strains that were indicative of different species.

Furthermore, the average nucleotide identity (ANI) index revealed that strain B2D shared the highest values with the strains *E. anophelis* subsp. *endophytica* JM-87^T at 98.91% and *E. anophelis* subsp. *anophelis* R26^T at 97.35% (Figure 5). The higher than species threshold values (≥95% pairwise ANI value) delineated strain B2D into *E. anophelis* based on its high genomic coherence with the mentioned *E. anophelis* reference strains. In addition, strain B2D shared lower than species threshold values (<95% pairwise ANI values) with all the other reference *Elizabethkingia* strains (Figure 5). Combining the results, it thus provided strong support for the taxonomic placement of strain B2D, indicating that it is a strain of *E. anophelis*.

3.5. Comparative Genomic Analysis

The genome length of most *Elizabethkingia* species ranged from approximately 3.8 Mbp to 4.4 Mbp, with the total number of genes ranging from 3,522 to 4,113, except for *E. argenteiflava*. Specifically, strain *E. argenteiflava* YB22^T that was isolated from soybean pod exhibited a significantly smaller genome length of 2.7 Mbp and a total number of 2,649 genes (Table 3).

Functional annotation of all the genes in the genomes of these reference strains of different *Elizabethkingia* species revealed highly similar distribution patterns across different COG categories, with one notable exception of strain *E. argenteiflava* YB22^T. Among the reference strains of different *Elizabethkingia* species, approximately 25% of the genes were not associated with known functions. The COG categories with the highest number of genes in these reference strains were transcription (K), cell wall/membrane/envelope biogenesis (M), and amino acid transport and metabolism (E) (Figure 6).

In contrast, the genome of strain *E. argenteiflava* YB22^T exhibited a lower proportion of genes with unknown functions at 17.6%. Approximately 18.5% of the genes in the genome of this strain were assigned to the COG category replication, recombination, and repair (L), followed by cell wall/membrane/envelope biogenesis (M), and amino acid transport and metabolism (E) (Figure 6).

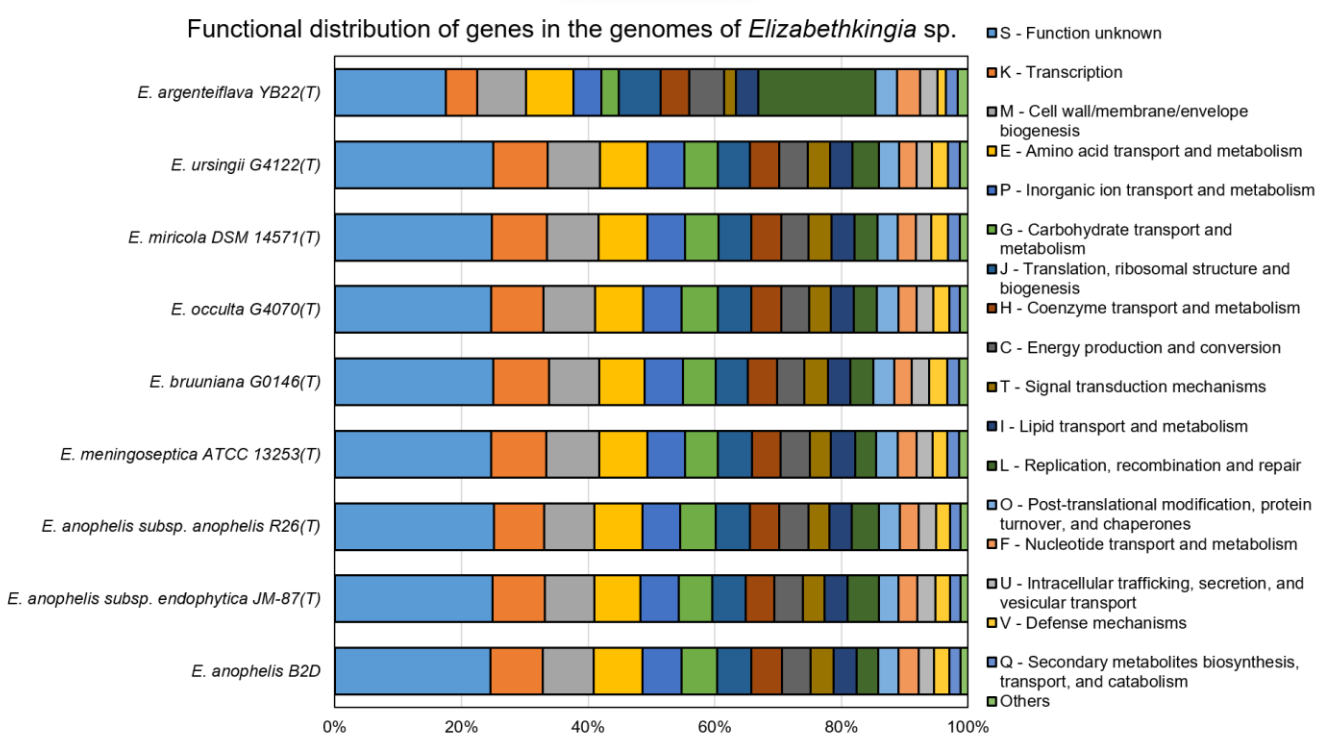

Figure 6. Functional distribution of genes in the genomes of strain B2d and type strains of *Elizabethkingia* species into different COG categories.

3.6. Comparative resistome analysis

Strain B2D harboured multiple antibiotic resistance genes, such as *blaCME-1*, *blaGOB-40*, and *blaB-14* that were encoded for enzymes known as beta-lactamases (Table 4). These genes could potentially confer resistance in strain B2D to beta-lactam drug classes, including cephalosporins, penems, and carbapenems. This finding helped explain its high level of MICs toward antibiotics such as penicillin, penicillin with beta-lactamase inhibitors, first to fourth-generation cephalosporins, and carbapenems (Table 2).

A rigorous RGI search against the CARD database identified the presence of at least one strict or perfect hit of a beta-lactamase-encoding gene in strain B2D and all the reference strains of different *Elizabethkingia* species, except for strain *E. argenteiflava* YB22^{T} (Table 4). This suggested that most members of the genus potentially exhibited intrinsic resistance towards beta-lactam antibiotics. Apart from *E. argenteiflava* YB22^{T}, strain B2D and all the reference *Elizabethkingia* strains possessed *adeF* and *wanW* genes that were potentially responsible for antibiotic efflux and glycopeptide resistance, respectively. Of particular note, the RGI analysis unveiled the presence of *qacJ* or *qacG* genes that were responsible for antibiotic efflux, as well as *vanT* that was responsible for antibiotic target alteration, thus conferring resistance towards glycopeptide antibiotics in strain B2D and all reference strains of *Elizabethkingia* (Table 4).

Table 4. Antibiotic resistance genes in strain B2D and type strains of *Elizabethkingia* species that were scored as strict and perfect hits with reference to CARD.

Drug class	**AMR gene family**	**B2D**	**Endophytica**	**Anophelis**	**Bruuniana**	**Occulta**	**Meningo**	**Miricola**	**Argen**	**Ursingii**
Penam	CME beta-lactamase	*blaCME-2*	*blaCME-2*		*blaCME-2*			*blaCME-1*		
Carbapenem; cephalosporin; penam	GOB beta-lactamase	*blaGOB-40*	*blaGOB-43*	*blaGOB-20*	*blaGOB-29*	*blaGOB-35*		*blaGOB-32*		*blaGOB-37*
Carbapenem; penam	BlaB beta-lactamase	*blaB-14*	*blaB-11*	*blaB-18*	*blaB-22*	*blaB-27*	*blaB-19*	*blaB-26*		*blaB-8*
Disinfecting agents and antiseptics	Small multidrug resistance (SMR) antibiotic efflux pump	*qacG*	*qacG*	*qacG*	*qacG*	*qacG*	*qacJ*	*qacG*	*qacJ*	*qacG*
Fluoroquinolone antibiotic; tetracycline antibiotic	Resistance-nodulation-cell division (RND)	*adeF*	*adeF*	*adeF*	*adeF*	*adeF*	*adeF*	*adeF*		*adeF*

Drug class	AMR gene family	B2D	Endophytica	Anophelis	Bruuniana	Occulta	Meningo	Miricola	Argen	Ursingii
	antibiotic efflux pump									
Glycopeptide antibiotic	Glycopeptide resistance gene cluster	*vanT*	*vanT*	*vanT*	*vanT*	*vanT*	*vanT*	*vanT*	*vanT*	*vanT*
	Glycopeptide resistance gene cluster	*vanW*	*vanW*	*vanW*	*vanW*	*vanW*	*vanW*	*vanW*	*vanW*	*vanW*

B2D – *E. anophelis* B2D; Endophytica – *E. anophelis* subsp. *endophytica* JM-87^T; Anophelis – *E. anophelis* subsp. *anophelis* R26^T; Bruuniana – *E. bruuniana* G0146^T; Occulta – *E. occulta* G4070^T; Meningo – *E. meningoseptica* ATCC 13253^T; Miricola – *E. miricola* DSM 14571^T; Argen – *E. argenteiflava* YB22^T; Ursingii – *E. ursingii* G412

4. Discussion

In this study, strain B2D isolated from the dental plaque of a patient was determined to be *E. anophelis* through 16S rRNA and core genes-based phylogenetic analyses, as well as calculation of ANI, an overall genome-relatedness index. *E. anophelis* is among the three *Elizabethkingia* species that frequently cause human infections, in addition to *E. meningoseptica* and *E. miricola* [38]. The isolation of *E. anophelis* from dental plaque or human oral cavity is unprecedented. This genus has not been described as oral microbial taxa in the expanded Human Oral Microbiome Database (eHOMD). Although there have been no previous reports of *E. anophelis* in the oral cavity of patients, *E. miricola* has been identified as an opportunistic pathogen that infected the oral cavity of an immunocompromised patient [39]. These findings suggested that strain B2D may represent a potential opportunistic pathogen within the human oral cavity.

Elizabethkingia species had been reported to be prone to misidentification using common microbial identification systems such as VITEK 2, API systems, and MALDI-TOF mass spectrometry [11]. This misidentification issue is particularly evident in clinical settings, where *E. anophelis* is often misidentified as *E. meningoseptica* [11]. To address this issue, this study employed molecular approaches including genomic and phylogenetic analyses for accurate taxonomic identification of strain B2D. The use of molecular methods such as 16S rRNA gene and core genes-based phylogenetic analyses, and overall genome relatedness are able to provide consistent results and higher precision in taxonomic identification of strain B2D. Furthermore, the use of whole genome sequences has played an increasingly important role in the taxonomic delineation of *Elizabethkingia* strains. This has led to the recent description of three new species, namely *E. bruuniana*, *E. ursingii* and *E. occulta* [4]. As whole genome sequencing data will be able to provide more accurate and comprehensive taxonomic classifications, it should be incorporated in future studies of the genus *Elizabethkingia* [40].

Among the most prevalent antibiotic resistance genes detected in all the *Elizabethkingia* strains in this study were *qacG* and *qacJ* which are known to confer reduced susceptibility to cationic antiseptic agents such as benzalkonium chloride [41], and *vanT* is one of the genes that confer glycopeptide resistance (Table 4). Except for *E. argenteiflava* YB22^{T}, all the *Elizabethkingia* strains included in this study harbour beta-lactamase gene(s) in their genome. We postulate that these genes might be lost in between the contigs of the genome of *E. argenteiflava* YB22^{T} which was fragmented into 689 contigs [2]. Resistance to beta-lactams is commonly reported in *E. anophelis* [9, 10]. Our resistome analysis reveals the presence of various *bla*$_{B}$ and *bla*$_{GOB}$ genes in the genomes of strain B2D and the other *E.*

anophelis type strains, thus suggesting their roles as the responsible resistance genes towards beta-lactam antibiotics including cephalosporins and carbapenems. Notably, these groups of beta-lactamases are also the most prevalent antibiotic resistance genes among the type strains of the other *Elizabethkingia* species (Table 4). In addition, strain B2D, *E. anophelis* subsp. *endophytica* JM-87^T and two other species also co-carry the gene *bla*$_{CME}$. These observations warrant concern about the use of antibiotics as carbapenems are among the beta-lactams that are the last line of antibiotics used for bacterial treatment [42, 43]. These findings provide additional evidence that resistance to beta-lactam antibiotics could be intrinsic to *Elizabethkingia* species [38].

In conclusion, this study provided a comprehensive taxonomic identification and genomic analysis of strain B2D that was isolated from a dental plaque sample. Through the application of molecular methods, including 16S rRNA gene and core genes-based phylogenetic analyses, and calculation of the overall genome relatedness index, strain B2D is classified as *Elizabethkingia anophelis*. The multidrug-resistant phenotype of strain B2D as revealed and confirmed by the whole genome analysis indicates its potential to act as a reservoir of beta-lactamase genes. In addition, its presence on dental plaque within the human oral cavity indicates that this strain could be an opportunistic pathogen that might harm the health of immunocompromised patients. Except for *E. argenteiflava* YB22^T with significant variation, genomic analysis on strain B2D and all the type strains of *Elizabethkingia* species reveal highly similar distribution of genes in their genomes into different COG categories. Resistome analysis reveals prevalent antibiotic resistance genes among the studied strains, suggesting that the resistance towards beta-lactam antibiotics in *Elizabethkingia* species could be intrinsic to the genus. These findings serve as the foundation for a deeper understanding of the resistome of *Elizabethkingia* species, particularly in the context of emerging antibiotic resistance and its implications for clinical management and public health [44].

Acknowledgement: Kok-Gan Chan thanks the financial support from the University of Malaya (grants numbers: ST006-2024; and UM-MOHE HIR Grant UM.C/625/1/HIR/MOHE/CHAN/14/1, no. H-50001-A000027; UM-MOHE HIR Grant UM.C/625/1/HIR/MOHE/CHAN/01, No. A000001-50001).

Authors' Contribution: KGC conceived the ideas and designed the experiments. KGC, WFY and HSY contributed resources. SYG, KOC, YJML, CYC, YYL and KWH performed the experiments and data analysis. SAK and NHAK obtained the dental samples and applied the ethics approval. All authors prepared, read, and approved the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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