Infection and Drug Resistance

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ORIGINAL RESEARCH

Whole Genome Sequencing of First *Janibacter indicus* Isolate in China Revealed Three Unique Genomic Islands Compared with Saprophytic Strains

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Introduction: Janibacter caused bacteriemia is one of the rare infections.

Methods: In the present study, we report the first isolation of *Janibacter*, a rare bacterial infection, from a bacteremia patient in China. Its 16S rDNA was amplified and designated as *Janibacter* YFY001, which belongs to *J. indicus*. In addition, its genome was sequenced through combined second- and third-generation genome sequencing methods.

Results: Based on its genome, we identified many virulence factors, such as catalase, gelatinase, FbpABC systems, and resistant genes, among others. Interestingly, three genomic islands were found in YFY001 by comparing its genome to environmental *Janibacter* strains.

Discussion: Our study not only provides the necessary genomic information for in-depth study of *Janibacter*, but also provides a novel methodology for studying future cases of rare bacterial infection.

Keywords: bacteriemia, Janibacter, genomic sequencing

Introduction

Rare bacterial infection represents a diverse group of bacterial species with ≤10 clinical reports in the PubMed database.[1] Patients infected with rare bacteria likely acquire infection through environmental exposure, including medical exposure, as most of them commonly reside in the natural environment; nonetheless, a small number of reports indicate that these bacteria infrequently cause human infections. Indeed, some *Janibacter* species cause rare bacterial infections, with three clinical cases reported to date. In 2005, two different groups isolated two distinct species of *Janibacter* from patients at the same time.[2–4] In 2015, a research group in Spain reported their first finding of *J. terrae*-infected human blood.[4] *Janibacter* is difficult to identify using morphological characteristics and biochemical reactions, instead requiring rRNA sequencing and phylogenetic analysis of positive culture samples. Meanwhile, it is important to acquire an initial diagnostic report as *Janibacter* is resistant to many antibiotics.

The genus *Janibacter* contains gram-positive aerobic cocci to rod-like bacteria that are non-spore-forming, non-motile, and lack mycelia. Additionally, these bacteria are catalase-positive, urease-negative, and oxidase-variable. Its colonies are smooth, circular, and usually yellow.[5] Optimal growth occurs at 30 degrees Celsius. *Janibacter* has

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a unique complex fatty acid profile and lacks mycolic acids. The G+C content of its DNA is approximately 70 mol%. Until now, *Janibacter* could be divided into nine strain types: *J. limosus, J. terrae, J. indicus, J. melonis, J. anophelis, J. hoylei, J. corallicola, J. alkaliphilus*, and *J. cremeus*.[5–13] *J. indicus* was first isolated by Zhang et al in the Indian Ocean. The phylogenetic tree of *J. indicus* and other Janibacter strains was constructed by 16s rRNA, and revealed that *J. indicus* is related to *J. terrae, J. cremeus, J. anophelis, J. hoylei, J. limosus, J. corallicola, J. melonis*, and *J. alkaliphilus* (Zhang et al.). Most of these strains exist only in the natural environment, except for *J. terrae* and *J. melonis*, which have previously been isolated from patients. Nonetheless, we isolated *J. indicus* from a bacteremia patient in the present study and thereby demonstrated that this strain could also cause host infection.

Second-generation sequencing (SGS) technologies have promoted the characterization of microbial genomes; however, drawbacks in SGS methods have also hindered this process. SGS produces a huge amount of short length reads which can be difficult to assemble into a complete genome. Therefore, many genomes are incomplete and are instead submitted as draft genomes—sometimes with hundreds of contigs which could provide limited information. Alternatively, third-generation sequencing methods—such as the Pacific Biosciences (PacBio) single-molecule real-time (SMRT) sequencing platform—can provide reads more than 3 kb long. These longer reads can contain the repeat region, and thus facilitate the generation of complete genomes without the need for additional sequencing. Subsequently, many of these genomes could help to carry out comparative analysis, functional, virulence, and antibiotic resistance studies.

Recently, we isolated and identified the first *Janibacter* strain in China from the blood of a patient who suffered from a viral central nervous system (CNS) infection with secondary sepsis. In order to identify the virulence factors underlying disease, a greater understanding of this bacteria at the genomic level is required. Therefore, we attempted to complete the genome of this strain; however, the high GC content of *Janibacter* prevented complete genome assembly using SGS methods. Thus, we combined SGS (Hi-seq) and third-generation sequencing (Pacific Bio method) techniques to assemble the completed genome. Finally, we compared the genomes of our isolate and two other environmental strains to identify the resistance genes and virulence factors of *Janibacter*.

Materials and Methods

Strain

Janibacter YFY001 was isolated from the blood of a patient who was diagnosed as having an unknown viral CNS infection with secondary sepsis. Written informed consent for the publication of their details was obtained from the study participant. A single colony was recovered from blood agar (5% sheep blood with Colombia agar, Oxoid) and maintained in nutrient broth (Oxoid). This study was approved by the First Affiliated Hospital of Nanchang University (approval no. 2014036).

Bacterial Identification and in vitro Drug Susceptibility Tests

The patient's blood was inoculated into aerobic and anaerobic bottles and cultured using the BACTEC FX system. After positive alarm, the media was transferred onto blood agar, China blue agar, and chocolate agar with 0.2% (10μg/mL) Vancomycin. After being cultured for 48 to 96 hours at 35°C, bacterial isolate was identified using the Vitek 2 Compact System (BioMérieux Clinical Diagnostics) and the MicroScan WalkAway 96 System. Antibiotic susceptibility tests were performed following the standard *S. aureus* procedure provided by the CLSI in 2020.[14]

Bacterial DNA Extraction, 16S rDNA Sequencing, and Phylogenetic Analysis

Janibacter YFY001 was grown in nutrient broth (Co. Oxford) for 96 hours at 37 degrees Celsius before genomic DNA was extracted using a QIAamp DNA Mini Kit. Subsequently, 16S rDNA amplification was carried out using the universal 16S rDNA primers 27f (AGAGTTTGATCMTGGCTCAG) and 1492r (GGTTACCTTGTTACGACTT).[15] The amplification product was sequenced using the Applied Biosystems 3730 platform. After sequencing, contigs were assembled and blasted using the NCBI website (http://www.ncbi.nlm.nih.gov/) using the 16S ribosome RNA database.

To elucidate the phylogeny between our isolate and remaining strains, 16S rDNA sequences were collected and MEGA6 was used for the phylogenetic analysis.

Genome Sequencing

Janibacter was isolated from the patient's blood and stored in our hospital. Genomic DNA for *Janibacter* was extracted using a Qiagen MagAttract HMW DNA Kit (QIAGEN 67563). For PacBio *RS* sequencing (Menlo Park, CA), a total of 5 μg of genomic DNA was sheared by g-TUBE (Covaris,

US) and a library of 10 kb sequences was constructed using the standard PacBio *RS* sample preparation instructions for sequencing on Pacific Biosciences *RS* II (Menlo Park, CA) platforms. In addition, a 300 bp paired end library was prepared according to the Illumina TruSeq DNA sample preparation recommendations and was sequenced on HiSeq 2500 platforms with read lengths of 150 bp.

Genome Assembly and Annotation

The PacBio data (one single-molecule real-time SMRT cell, 3975 bp average read length, approximately 53× coverage) were assembled using the Hierarchical Genome Assembly Process (HGAP) software,[16] resulting solely in one-contig assembly. The HiSeq data (approximately 300× coverage) were mapped against the assembly to proofread the PacBio data using Bowtie2[17] and SAMtools.[18] Finally, a whole genome assembly without redundancy was obtained.

Gene prediction was performed using Glimmer 3.02.[19] tRNA and rRNA genes were predicted by tRNAscan-SE[20] and RNAmmer,[21] respectively. Predicted genes were blasted in a non-redundancy (nr) database on NCBI and annotated. Pathways involved in the genes were constructed using the Kyoto Encyclopedia of Genes and Genomes (KEGG).[22] Gene COG were classified according to a conserved domain database.[23]

Comparative Genomics

Mauve 2.3.1 (http://darlinglab.org/mauve/user-guide/introduction.html), Blast-2.2.30+, PATRIC, and Perl Scripts were used for comparative genomic studies.

Nucleotide Sequence Accession Numbers

SubmissionID: SUB1159744

BioProject ID: PRJNA300616

Results

Case Report

A 61-year-old woman presented to the Emergency Department at the First Affiliated Hospital of Nanchang University on 7 July 2014. Her family members explained that she had developed a fever with headaches, chills, dizziness, and body aches after a rainstorm and had lost consciousness two day later, resulting in hospitalization. Upon physical examination, she was found to be afebrile with a heart rate of 88 bpm, blood pressure of 124/77 mm Hg, and a respiratory rate of 22 breaths per minute. Subsequent examination of the patient's head, heart, lungs, and abdomen yielded normal results. Kernig's sign was positive on both sides of the body. Babinski and Brudzinski signs were both negative. On 8 July, a computed tomography (CT) scan of the head and abdomen also returned normal results. Chest CT scans revealed pleural effusion on both sides and mild lung infection. Laboratory results—including routine complete blood count, hepatic function, coagulation function, and cardiograph—were all normal. On 9 July, a CSF chemical study indicated normal glucose and chloride levels and a protein level of 1913 mg/L. On 10 July, ESR was 46 mm/h. The ER physicians concluded that she had a central nervous system infection, viral meningitis, and lung infection. No improvement was seen after four days of anti-infective, mannitol, and fluid infusion treatment, and the patient was transferred to the Department of Infectious Diseases (DID) on 11 July. At the DID, she was treated with mannitol, dexamethasone, and Rocephin, as in the ER, but with the addition of acyclovir. On 14 July, the patient had a fever with a body temperature of 39 degrees Celsius. Laboratory studies indicated a leukocyte concentration of 10.18E9/L and a neutrophil concentration of 6.92E9/L. Blood culture was sent to the Department of Laboratory Medicine (DLM) and the patient's antibiotic was changed to Tazocin. The patient then became well the next day. On 18 July, the DLM reported that they had cultured gram-positive bacteria. Dermacoccus nishinomiyaensis and susceptibility results were reported on 24 July before the patient was finally discharged on 4 August (Figure 1).

Bacterial Isolation and Identification and Antibiotic Susceptibility Test

Aerobic and anaerobic blood cultures were directed to the DLM. After almost five days of culture, the equipment presented a positive alarm for the aerobic bottle. After another 48 hours of culture, wet yellow colonies could be seen only on blood agar. Gram staining indicated a gram-positive coccoid bacteria. An identification report obtained with the VITEK 2 Compact System indicated that the isolate was *Dermacoccus*. In addition, we analyzed the isolate using the MicroScan WalkAway 96 system, where it was identified as "Micrococcus", a different result to that of the VITEK 2 Compact System report. Subsequently, we confirmed the catalase and oxidase activity of the isolate. However, the isolate exhibited catalase-positive and oxidase-negative

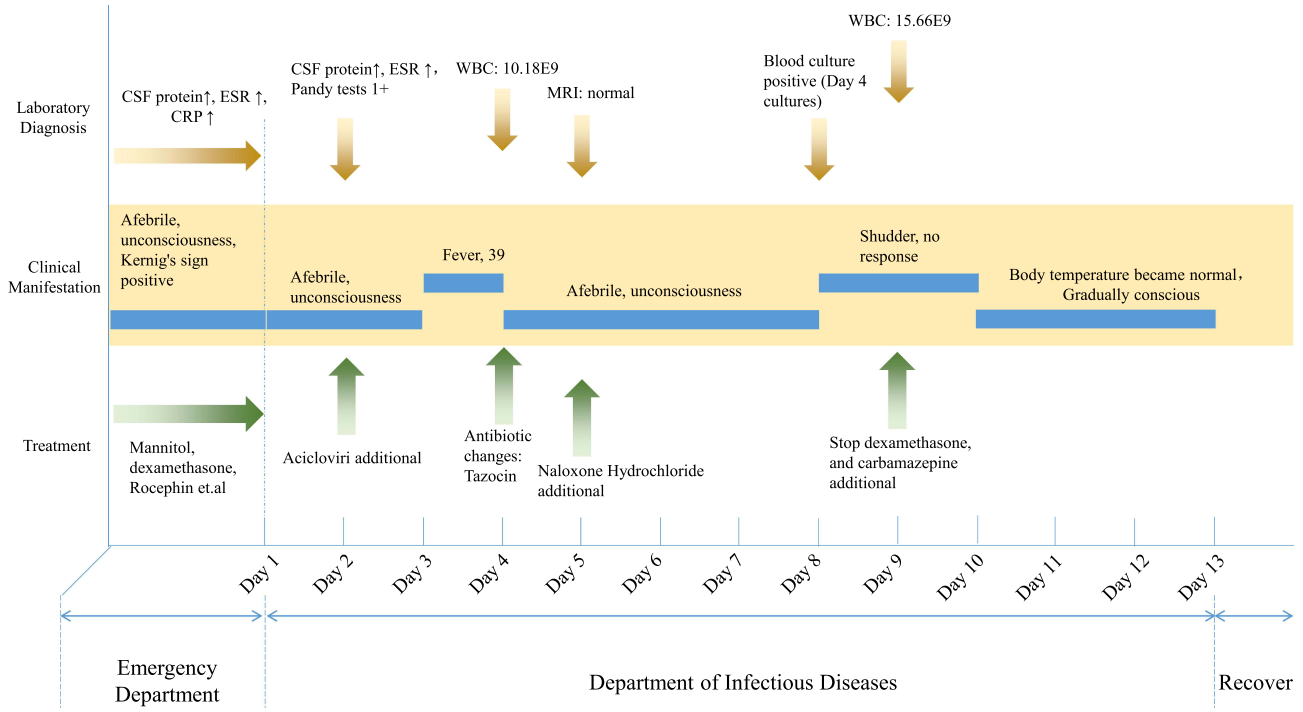

Figure 1 Timeline of the patient's illness and treatment in the hospital. Time in days is shown along the x axis with treatment, clinical manifestation, and laboratory diagnosis along the y axis. The patient was sent to the Emergency Department on Day 1 and transferred to the Department of Infectious Diseases in the following days. We reported a positive blood culture result on day 8.

reactions, which differs from *Dermacoccus*. Therefore, partial 16S rDNA sequencing and Blast were carried out on our isolate, revealing a 99.8% match to *Janibacter*.

Additionally, antimicrobial susceptibility to 20 antimicrobials was determined using MicroScan plates which were incubated in air at 35 degrees Celsius and read after 72 hours. The susceptibility results were performed according to CLSI M100 standards.[14] Based on the anti-drug susceptibility test results, *J. indicus* may be resistant to rifampin, clindamycin, and ceftazidime. In addition, compared with *J. terrae, J. indicus* was sensitive to piperacillin (Table 1).

De Novo Assembly of *Janibacter* YFY001 Using the PacBio *RS* Platform and Illumina HiSeq

To identify any possible virulence and antibiotic resistant factors at the genetic level, the whole genome of *Janibacter* YFY001 was sequenced. We combined second- and third-generation sequencing methods to complete the genome using the Illumina and PacBio platforms, respectively. Using one single-molecule real-time sequencing (SMRT) cell, 64,983 PacBio subreads totaling 180,137,073 nucleotides were obtained with a mean length of 3772 bp. Meanwhile, using the Illumina HiSeq platform, 3,969,587 paired reads totaling 1,198,815,274 nucleotides were recovered with a mean length of 300 bp after trimming and quality filtering.

One single contig was produced for *Janibacter* YFY001 with the automated gap closing tool FGAP using only PacBio subreads (Figure 2A). A whole genome of 3,401,190 bp was produced, comprising 3455 predicted open reading frames (ORFs), with a G+C content of 71.24%. The genome has two predicted copies of 5S, 16S, and 23S rRNA genes and 47 predicted tRNAs. There are putative genes for complete glycolysis, the tricarboxylic acid cycle, pentose–phosphate pathways, and predicted genes for the utilization of fructose, sucrose, and galactose. Of the annotated ORFs, 772 hypothetical proteins, 3326 proteins with COG assignments (Figure 2B), and 913 proteins with pathway assignments were identified. There are 65 predicted ABC transporters (or ABC transporter-related proteins). The genome contains Sec-dependent pathway proteins, including SecY, Sec D/F, YidC, SecA, Ffh, and FtsY. Signal peptidase I and II could also be found in the genome. All the essential amino acid genes could be predicted in the genome. In addition, there are two predicted phage-related proteins: gp37 and gp43.

Table 1 Comparison of Biochemical Reactions and AST Results Between YFY001 and Other Janibacter

	Janibacter **YFY001**	*J. brevis*	*J. terrae*			
			CNM582-06	**CNM586-06**	**CNM588-06**	**CNM654-06**
Characteristic						
Catalase	+	+	+	+	+	+
Nitratase	-	-	+	+	+	+
Pyrazinamidase		+	+	+	+	+
Alkaline phosphatase		+	+	+	+	+
A-glucosidase	-	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+
Maltose Assimilation			+	+	+	+
Oxidase	-	-	-	-	-	-
Assacharolytic			-	-	-	-
N-acetylglucosamine			-	-	-	-
Phenylacetic acid			-	-	-	-
Urease	-	-				
Indole	-	-				
Antimicrobial						
Benzylpenicillin	N/A*	0.5	2	1	2	2
Ampicillin	2	N/A	8	4	8	8
Amoxicillin	N/A	0.38	3	2	3	3
Piperacillin	≤16	0.38	>256	>256	>256	>256
Cefepime	8	8	N/A	N/A	N/A	N/A
Cefotaxime	≤2	4	4	3	4	3
Ceftazidime	>16	N/A	>256	>256	>256	>256
Imipenem	≤1	N/A	0.125	0.19	0.19	0.25
Vancomycin	≤0.25	0.38	0.5	0.5	0.5	0.5
Teicoplanin	N/A	0.25	0.75	0.75	0.75	1
Linezolid	≤1	N/A	0.5	0.75	0.5	1
Daptomycin	0.5	N/A	0.064	0.064	0.064	0.064
Tetracycline	≤4	N/A	1.5	1.5	1	3
Ciprofloxacin	≤1	0.25	0.25	0.19	0.25	0.25
Moxifloxacin	≤0.5	N/A	0.064	0.047	0.064	0.047
Levofloxacin	≤1	N/A	0.25	0.19	0.25	0.38
Erythromycin	≤0.5	N/A	0.5	0.38	0.25	0.25

(*Continued*)

Table 1 (Continued).

	Janibacter YFY001	*J. brevis*	*J. terrae*			
			CNM582-06	CNM586-06	CNM588-06	CNM654-06
Clindamycin	>4	N/A	3	6	6	6
Gentamicin	≤4	0.75	1.5	1.5	2	3
Tobramycin	≤4	0.5	2	3	2	4
Amikacin	≤8	N/A	0.75	0.75	1	0.5
Rifampin	>2	N/A	>32	>32	>32	>32

Note: *These data was not provided.

Identification of Possible Virulence Factors Using Genomic Information

Gelatinase Gene (+orf2875)

Gelatin hydrolysis tests showed that *Janibacter* could degrade gelatin and a putative gelatinase was identified in the genome. As gelatinase (GelE) has been mostly studied in Enterococcus,[24] we blasted the GelE sequence of Enterococcus from NCBI against theYFY001 genome and found a homologous protein of GelE, designated as orf02875 (JaniGelE). Using a conserved domain search engine, we

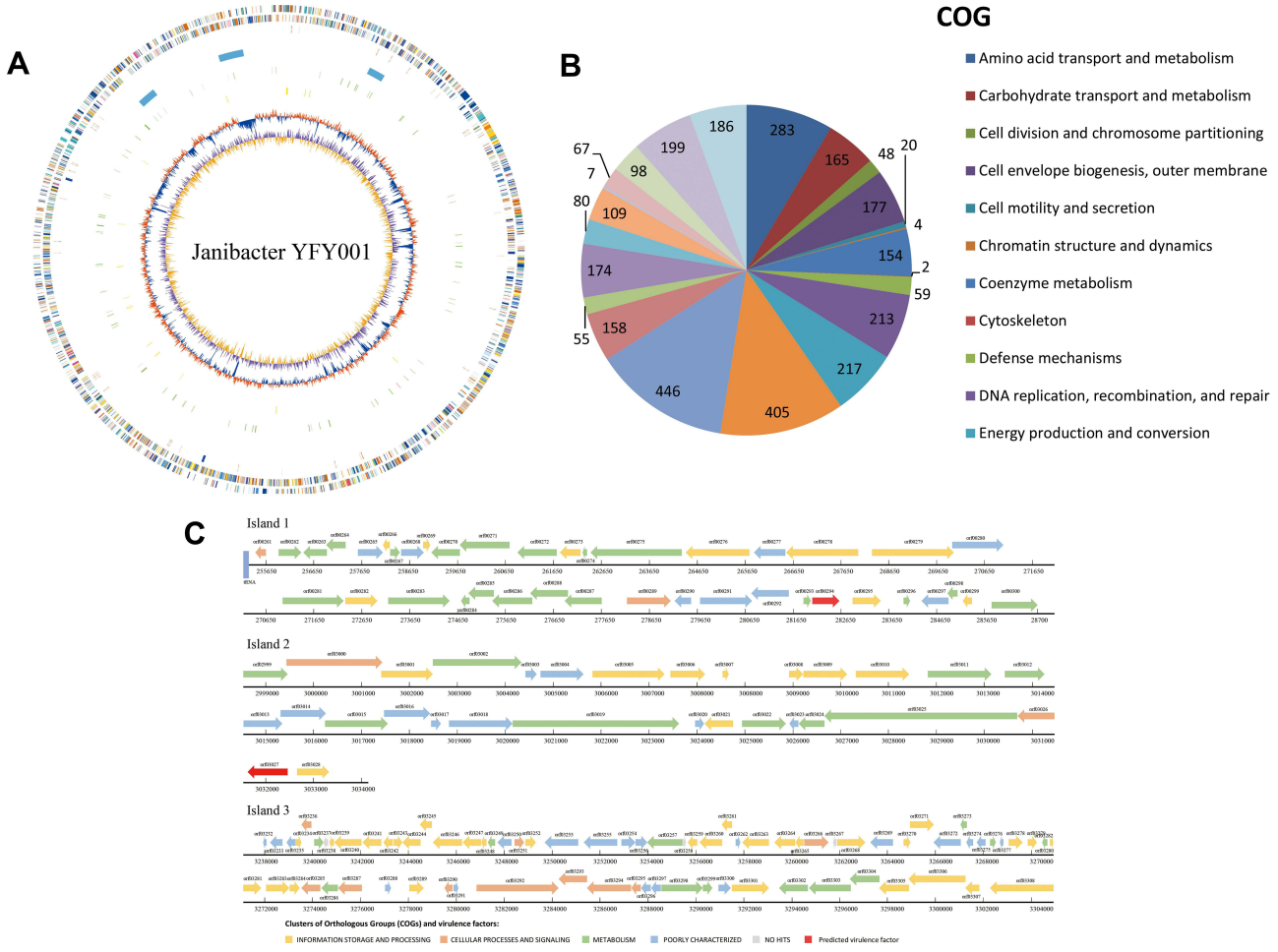

Figure 2 Genomic analysis of *Janibacter* YFY001. (**A**) Circular representation of the Janibacter YFY001 genome with predicted CDSs. Circles range from 1 (outer circle) to 8 (inner circle). Circles 1 and 2, genes on forward and reverse strands; circle 3, tRNA genes; circle 4, genomic island; circle 5, predicted resistance genes; circle 6, rRNA genes; circle 7, GC bias ((G-C)/(G + **C**); red indicates values >0; blue indicates values <0); circle 8, G+C content. (**B**) COG analysis of *Janibacter* YFY001 genome. (**C**) Genomic islands in *Janibacter* YFY001. Different colors represent different COGs.

found that orf02875 has an M4 neutral protease domain, and a LasB (also known as Zn-dependent metalloprotease) domain, which is similar to the structure of gelatinase. GelE belongs to the M4 family of bacterial zinc metalloendopeptidases.[25] Metalloproteases are the most diverse of the four main types of protease, with more than 50 families identified to date. In these enzymes, a divalent cation, usually zinc, activates the water molecule. It always contains an "abXHEbbHbc" structure, where "a" is most often valine or threonine and forms part of the S1' subsite in thermolysin and neprilysin, "b" is an uncharged residue, and "c" is a hydrophobic residue. JaniGelE has a similar structure to GelE.

Catalase Gene (orf01884)

During clinical diagnosis, YFY001 was found to be catalase positive. Previous studies revealed that almost all of the Janibacter strains exhibit a positive reaction in catalase tests. Consistent with these findings, we were able to identify a catalase gene (orf01884) in the YFY001 genome.

Virulence Factors

In order to determine the possible virulence factors in *Janibacter* YFY001, we blasted the genome using the VF database[26] with E-value 1e-5, identity 20%, and coverage 50%. The results indicated 82 possible virulence factors for YFY001 (Table S1).

Antibiotic Resistant Genes

According to the antibiotic susceptibility test, YFY001 demonstrated resistance to certain drugs. Therefore, Resistance Gene Identifier VERSION 2 was used for the selection of resistant genes, and 16 possible resistant genes were found in the YFY001 genome.[27] They belong to five different COG categories, including carbohydrate transport and metabolism; cell envelope outer-membrane biogenesis; defense mechanisms; DNA replication, recombination, and repair; and signal transduction mechanisms.

Previous studies indicated that most *Janibacter* were resistant to Rifampin, but they did not elucidate the mechanism of resistance. Rifampin was previously shown to be active in vitro against most *Micrococcus*.[28] Until now, studies which focused on Rifampin resistance mechanisms demonstrated that it was related to rpoB mutations.[29] A RIF-resistance-determining region (RRDR), which indicates that mutations occur in an 81 bp region of cluster I, was found across all bacterial species.[29] Here, we compared the sequence of rpoB among YFY001, *E. coli*, and Mtb and determined that there was an amino acid mutation in this RRDR region (S443N). This novel mutation has not been reported before and might be responsible for the RIF resistance seen in *Janibacter*.

Genomic Comparison Indicates That YFY001 Has Three Possible Genomic Islands

In addition to YFY001, two other *Janibacter* genomes have been sequenced previously. Information on these three strains is presented in Table 2. Comparative genomic analysis was carried out using BlastP with E-value 1e-5, BBH identity 20%, and coverage 50%, and 1009 core orthologs could be found in all three strains (Figure 3A). Additionally, 985 specific orthologs could only be found in YFY001, which could be mapped to carbohydrate metabolism; energy metabolism; lipid metabolism; nucleotide metabolism; amino acid metabolism; metabolism of other amino acids; glycan biosynthesis and metabolism;

Table 2 Basic Statistics of the *Janibacter* YFY001 Genome Sequenced in This Study, Compared with Those of *Janibacter* PVAS, and HTCC2649

	HTCC2649	Holey PVAS-1	YFY001
Isolate place	Stratosphere	Sargasso Sea	Human, blood
Genome size	4,221,782 bp	3,129,280 bp	3,402,770 bp
GC%		68.4%	71.24%
PATRIC total genes	4060	3464	3443
PATRIC CDS	4005	3101	3353
rRNA	4	9	6
tRNA	45	47	47
Genes with predicted function	2752	2003	2131

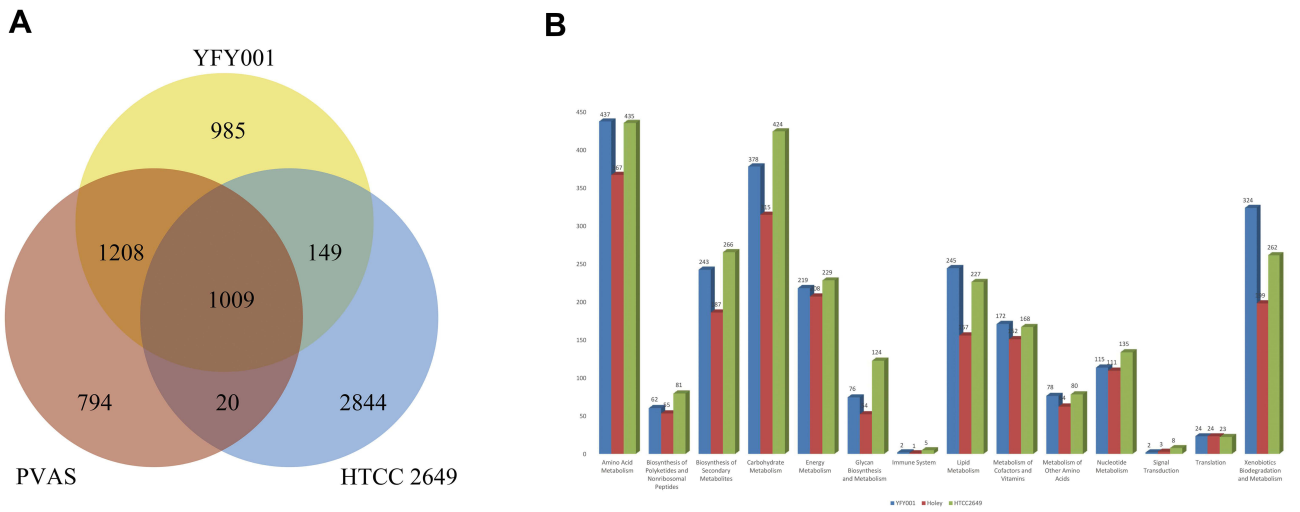

Figure 3 Comparative genomic analysis using *Janibacter* YFY001, PVAS, and HTCC 2649 genomes. (**A**) Venn figure of the comparative genomic results. (**B**) COG analysis of the comparison results.

metabolism of cofactors and vitamins; metabolism of terpenoids and polyketides; biosynthesis of other secondary metabolites; biodegradation and metabolism of xenobiotics; translation, replication, and repair; membrane transport; and signal transduction pathways (Figure 3B).

Interestingly, three specific regions—located in the 255,480–287,000 bp; 2,997,051–3,023,851 bp; and 3,238,801–3,302,701 bp regions—were found in genomic comparison results. Most of the genes in these areas are absent from environmental strains and thus may be inserted into the genome by horizontal gene transfer (HGT). In order to identify the genomic islands in YFY001, IslandViewer 3 and NCBI-Blast were both used. Finally, three probable genomic islands were found and designated as JaniIsland 1 (255,480–287,000 bp), JaniIsland 2 (2,997,051–3,023,851 bp), and JaniIsland 3 (3,238,801–3,302,701 bp). The structures of these three islands are presented in Figure 2C. In addition, we used VFDB, ARDB, and COG to predict the functions of the genes in these islands.[26,27] Subsequently, we considered that JaniIsland 1 may be a pathogenic island as it exhibits their classical structure, containing tRNA, virulence factors, etc.

Discussion

Identification and Taxonomy

Janibacter YFY001 was the first *Janibacter* strain isolated from a patient's blood in China. However, in our opinion, a number of *Janibacter* strains might be classified as other kinds of bacteria due to numerous complications which may arise during diagnosis. First of all, most hospitals use automatic chemical identification systems such as VITEK, MicroScan, and others. In the present study, both systems were used for detection. Using the VITEK-2 system, the isolate was initially identified as *Dermococcus* using a GP card, which can only identify 115 taxa of the most significant non-spore-forming, gram-positive bacteria. *Janibacter* is not on the list of identifiable bacteria for the VITEK 2 or the MicroScan 96 system, and therefore *Janibacter* infection is not considered. In addition, both *Dermacoccus/Kytococcus* and *Janibacter* belong to Actinomycetales, Micrococcineae, which may show similar biochemical reactions and lead to problems in identification. This indicates that many *Janibacter* infections may be misidentified as *Dermacoccus/Kytococcus* or *Micrococcus* due to the use of automatic identification systems. Therefore, we suggest that clinical isolates classified as *Dermacoccus/Kytococcus* or *Micrococcus* should undergo further 16S rDNA sequencing prior to final confirmation, which will provide physicians with more accurate results. Similar to most of *Janibacter spp., Janibacter YFY001* belongs to *J. indicus* and demonstrated catalase (+), oxidase (-), and gelatin hydrolysis (+) characteristics. The a-glucosidase activity was negative for *Janibacter YFY001*, which was different from *J. terrae* and *J. brevis* (Table 1).

Antibiotic Resistance-Related Genes

Antibiotic resistance is one of the key hazards of rare bacterial infections. At present, four different studies have indicated that

Table 3 Predicted Antibiotic Resistance Genes

Gene	Start	End	Strand	Length	Function	COG
orf00095	90,381	88,708	–	557	Drug resistance transporter, EmrB/QacA subfamily	Carbohydrate transport and metabolism
orf00111	103,973	105,481	+	502	Multidrug MFS transporter	
orf01253	1,259,782	1,258,376	–	468	Major facilitator transporter	
orf02206	2,210,762	2,211,919	+	385	Hypothetical protein	
orf02488	2,495,034	2,496,698	+	554	Major facilitator transporter	
orf03216	3,223,585	3,222,035	–	516	MFS transporter	
orf02345	2,352,909	2,351,734	–	391	Nucleotide sugar dehydrogenase	Cell envelope biogenesis, outer membrane
orf01710	1,710,193	1,708,307	–	628	ABC transporter	Defense mechanisms
orf02161	2,167,350	2,169,182	+	610	Multidrug ABC transporter ATPase	
orf00030	25,147	27,285	+	712	DNA gyrase subunit B	DNA replication, recombination, and repair
orf00031	27,357	29,927	+	856	DNA gyrase subunit A, partial	
orf02047	2,056,709	2,056,044	–	221	Two component system response regulator	Signal transduction mechanisms
orf02351	2,357,450	2,356,791	–	219	Chemotaxis protein CheY	
orf02382	2,388,275	2,388,952	+	225	Chemotaxis protein CheY	
orf02634	2,639,898	2,640,557	+	219	Two component transcriptional regulator	
orf02867	2,866,073	2,866,804	+	243	Transcriptional regulator	

Table 4 Similarities and Identities Between Different Bacteria with FbpABC Operon

Organism	Gene	A (S/I)	B (S/I)	C (S/I)
H. influenzae	Hit	50.4/31.7	52.8/31.3	56.9/36.4
S. marcescensb	Sfu	69.6/52.3	59.3/43.5	60.6/43.6
Y. enterocolitica	Yfu	71.2/52.9	58.4/41.7	62.6/42.1
N. gonorrhoeae	Fbp	54.8/33.4	54.1/30.8	55.6/37.4

Notes: Percent similar/identical residues (S, similarity; I, identity). Percent similar residues assuming that the following amino acid pairs are equivalent; I and V, S and T, E and D, K and R, F and Y.

Janibacter could be resistant to Ceftazidime, Clindamycin, and Rifampin. However, they did not elucidate the possible genes responsible for this resistance. In this study, we identified 16 genes that might be related to *Janibacter* resistance against these antibiotics, including many drug-resistant transporters, which would facilitate resistance to antibiotics (Table 3). In addition, the molecular mechanism of YFY001 resistance to RIF was found to most likely be caused by a mutation in the RRDR gene.

Pathogenicity and Genomic Islands

As very few studies have reported on the virulence factors of *Janibacter*, we were only able to predict possible virulence factors using bioinformatics-based methods. Initially, we identified *Janibacter* gelatinase, which might be related to *Janibacter* virulence. Through combined VFDB and genomic comparison results, we concluded that only 17 probable virulence factors existed in YFY001.

Iron acquisition plays an important role in the survival of pathogenic bacteria within the host. *Neisseria gonorrhoeae, Neisseria meningitidis*, and *Haemophilus influenza* can employ the FbpABC system (also called HitABC in *Haemophilus influenza*) for iron acquisition from hosts. Interestingly, we found a similar structure in YFY001 but not in the other two environmental strains. A *hit*ABC-like operon was found in the YFY001 genome and was designated as *jfu*ABC. The HitABC operon was confirmed to be an important factor for iron acquisition from hosts, which indicated that this system is essential for YFY001 survival in vivo. In addition, we compared the similarity and identity among these strains, as shown in Table 4.

Gene acquisition, gene loss, and other genomic alterations are important in the adaptive evolution of prokaryotes and are achieved by horizontal gene transfer and homologous recombination.[30] Over the past few decades, studies have shown that genomic islands (GEIs) are a new kind of rapid microbial gene exchange element.[30] Genomic islands are considered to be a way for microbes to adapt to the changing external environment, carrying pathogenic elements necessary for microbes and special

structures of metabolism-related components. When compared with other parts of microbial genomes, GEIs have an obvious difference in terms of their GC content. In this study, we predicted three GEIs within the genome of YFY001 by comparing it with two existing environmental *Janibacter* genomes. We found that most of the genes in the GEI components of YFY001 strains were absent from the environmental strains; however, the function of most of these genes remain unclear (Figure 2C). According to COG, most of the proteins in Island 1 contribute to metabolism while those of Island 3 plays a role in information storage and processing, implying that the islands would provide different functions during pathogenesis.

Furthermore, VFDB Blast results indicated that a virulence factor existed in Island 1, and that the structure of its components conformed to the basic structure of virulent islands. According to the genomic information obtained, we predict that Island 1 is a pathogenic island as it shows identical characteristics to classical pathogenic islands. In future studies, we intend to focus on the function of this pathogenic island.

Conclusion

In this study, we used genomic sequencing methods to elucidate the virulence mechanisms and possible resistance genes of *Janibacter YFY001*—a rare bacterial infection in humans. Our study provides a practical method for the study of rare clinical isolates and a way in which to obtain sufficient information to study the pathogenic mechanisms of rare pathogens. These techniques are expected to contribute to our understanding of rare pathogens and enable physicians to better treat patients affected by rare bacterial infections.

Ethics Statement

This study was approved by the First Affiliated Hospital of Nanchang University (approval no. 2014036).

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Disclosure

The authors report no conflicts of interest in this work.

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