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SHORT REPORT

Isolation and Characterization of a Novel Achromobacter Strain from a Diarrheal Stool Specimen

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Abstract: A novel bacterial strain, designated as L3024hy, was isolated from the fecal matter of a patient suffering from diarrhea in China. Whole-genome sequencing has identified this strain as a member of the genus *Achromobacter*. Comparative genomic analyses, including Average Nucleotide Identity (ANI) and digital DNA–DNA hybridization (dDDH), indicated that L3024hy constitutes a distinct lineage within this genus, as evidenced by ANI and dDDH values falling below established thresholds for species demarcation. This strain harbors multiple virulence genes associated with host colonization, suggesting its potential clinical significance. This study underscores the increasing diversity of the genus *Achromobacter* and emphasizes the necessity for further research on its role in human infections.

Keywords: whole-genome sequencing, average nucleotide identity, digital DNA-DNA hybridization, virulence-associated genes

Introduction

Achromobacter species, which belong to the family *Alcaligenaceae*, are non-fermentative gram-negative bacteria that have garnered attention because of their environmental resilience and clinical significance. These organisms are ubiquitous in various environments, including soil and water, and are known to be opportunistic pathogens, particularly in immunocompromised individuals.[1] The incidence of *Achromobacter* infections has been increasing, particularly among patients with cystic fibrosis, chronic lung disease, and those undergoing invasive procedures. The clinical management of *Achromobacter* infections poses significant challenges, as these bacteria often exhibit multidrug resistance, complicating treatment options and leading to increased morbidity and mortality rates.[2]

The classification of *Achromobacter* species is evolving, with several identified species including *Achromobacter xylosoxidans* and others.[3] However, the taxonomy of the genus is complex and unresolved, and further research is required to clarify the species distinctions and pathogenic potential. While traditional taxonomy relies on 16S rRNA gene sequencing, whole-genome sequencing (WGS) offers more precise species delineation using metrics, such as average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH).[4,5] These genomic tools are essential for distinguishing closely related species in genera such as *Achromobacter*, where phenotypic similarities often obscure taxonomic boundaries. Understanding *Achromobacter*'s biological traits and clinical implications of Achromobacter is vital for developing effective diagnostics and treatments given the rising prevalence of related infections.

This study details the isolation of strain L3024hy from a patient with diarrhea and its precise classification within the *Achromobacter* genus using whole-genome sequencing, ANI/dDDH analysis, and core-genome phylogenetics. We also

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evaluated its antimicrobial resistance and virulence genes to understand its clinical significance and clarify the genomic traits and potential impact of this new *Achromobacter* strain.

Methods

Strain Isolation

During routine checks for carbapenem-resistant bacteria at a tertiary teaching hospital, fecal samples were collected from diarrheal patients. On November 16, 2020, a sample from a 19-year-old Crohn's disease patient with a surgically treated perianal fistula revealed *Achromobacter sp*. strain L3024hy. The samples were incubated overnight in Brain Heart Infusion Broth and screened on MacConkey agar containing meropenem to detect resistant strains.

Whole-Genome Sequencing (WGS) and in silico Analyses

Genomic DNA was extracted using the SteadyPure Kit and sequenced on Illumina Novaseq 6000 and Nanopore PromethION platforms. For Illumina, DNA was sonicated to 350 bp, end-polished, A-tailed, and adapter-ligated, Fastp (0.23.1) assessed short-read sequencing quality by removing paired reads with adapter contamination, over 10% unknown bases, or more than 50% low-quality bases (Phred quality < 5), using default machine settings unless specified otherwise. The hybrid assembly strategy was implemented with Unicycler v0.4.8, which utilizes short reads to correct long reads, to obtain a high-quality, circularized genome sequence, and annotation was done with Prokka v1.14.[6,7] Abricate identified antibiotic resistance and virulence genes in the L3024hy strain using CARD and VFDB databases.[8,9]

Average Nucleotide Identity (ANI) and Digital DNA-DNA Hybridization (dDDH)

The genetic similarity of the isolated *Achromobacter spp*. strains was assessed using ANI and dDDH analyses. The complete genome sequences of other *Achromobacter spp*. in the NCBI database were used for comparison. ANI values were calculated using PyANI and dDDH estimates using the GGDC 2.1. These analyses aimed to evaluate whole-genome genetic similarity and determine taxonomic relationships between *Achromobacter spp*. isolates and related species, based on species delineation thresholds.

Phylogenetic Analysis

To determine the phylogenetic relationships between the isolated *Achromobacter* strains and the reference species, a phylogenetic tree was created using the complete genome sequences of 23 *Achromobacter* species from the NCBI database. The Snippy pipeline (v1.0) facilitated multiple sequence alignment and SNP calling. Core genome SNPs were used to construct a maximum-likelihood phylogenetic tree with Fasttree (v2.1), using the best substitution model from ModelFinder. The tree was visualized and annotated using iTOL (https://itol.embl.de/) and its robustness was assessed with 1000 bootstrap replicates.

Results and Discussion

On November 16, 2020, *Achromobacter sp*. strain L3024hy was isolated from a 19-year-old Crohn's disease patient with a perianal fistula. The carbapenem-resistant strain was identified as *A. xylosoxidans* by MALDI-TOF. Whole-genome sequencing, employing both long-read and short-read technologies (Table 1), was conducted for precise species identification. Long-read sequencing yielded 243,062 reads totaling 2.15 Gb, with an average read length of 8839.7 bp and N50 of 11,580 bp, indicating high continuity and reliability. Short-read sequencing generated 9.41 million raw reads (1.41 Gb), with 99.79% effective reads and excellent quality scores (Q30 = 89.95%, GC% = 66.21), ensuring the accuracy of further analyses. The hybrid assembly produced a single circular chromosome of 6,905,297 bp (GC% = 66) with 311× coverage, resulting in a fully closed genome (one contig, L50 = 1).

Based on the KmerFinder database, the strain L3024hy was classified as *A. insolitus*, however, classification based on the *nrdA* gene sequence (https://pubmlst.org/bigsdb?db=pubmlst_achromobacter_seqdef) identified it as *A. aegrifaciens* [n = 10], and this discrepancy was further compounded by variations in the mass spectrometry profiles. To accurately determine the phylogenetic position of strain L3024, we constructed a core-genome phylogenetic tree using an expanded dataset of 78 genomes. This included the 23 type strains of the genus *Achromobacter* and up to three additional genomes per species,

Table 1 Features of the Complete Whole-Genome Sequences of Strain L3024hy

Feature		
Long read	Number of Reads	243,062
	Total Bases (bp)	2,148,603,425
	Mean Read Length (bp)	8839.7
	N50 Read Length (bp)	11,580
	Mean Read quality	11.8
Short read	Raw Reads	9,409,762
	Clean Reads	9,389,616
	Raw Base (G)	1.41
	Clean Base (G)	1.41
	Effective (%)	99.79
	Q20 (%)	95.92
	Q30 (%)	89.95
	GC (%)	66.21
Assembly	Genome size (bp)	6,905,297
	Coverage (X)	311
	GC content (%)	66
	Number of contigs	1
	L50 value	1

offering a detailed view of phylogenetic relationships. (`ure 1A). Strain L3024hy clustered within the *A. aegrifaciens* clade but formed a distinct branch with high bootstrap support. This divergence was further quantified through ANI and dDDH analysis. Strain L3024hy had the highest ANI (94.76%) and dDDH (60.1%) values with the type strain of *A. aegrifaciens* (SAMEA6797398) (Table 2 and Figure 1B). An ANI value below 95% and a dDDH value below the 70% threshold confirmed that strain L3024hy represents a distinct and previously unrecognized species within the genus *Achromobacter*. Furthermore, an extensive ANI analysis of 583 *Achromobacter* genomes from NCBI database showed that only seven strains had an ANI value over 95%, confirming strain L3024hy as a distinct species (Table S1).

Table 2 Average Nucleotide Identity (ANI) and Digital DNA–DNA Hybridization (dDDH) Values Between Strain L3024hy and Type Strains of the Genus *Achromobacter*

Strain	Biosample	dDDH (%)	ANI (%)
Achromobacter aestuarii	SAMN10829291	20.7	76.14
Achromobacter agilis	SAMEA4780388	32.9	86.76
Achromobacter aloeverae	SAMN08715215	22.8	78.00
Achromobacter animicus	SAMEA6797393	28.6	84.40
Achromobacter anxifer	SAMEA6647246	41.6	90.35
Achromobacter arsenitoxydans	SAMN02469904	30	85.27
Achromobacter deleyi	SAMEA6647249	28.8	84.44
Achromobacter denitrificans	SAMEA3867444	33.3	86.96
Achromobacter dolens	SAMEA6797395	29.2	84.68
Achromobacter insuavis	SAMEA6797397	29.7	84.94
Achromobacter kerstersii	SAMEA2247603	28.6	84.17
Achromobacter marplatensis	SAMEA6647239	29.3	84.96
Achromobacter mucicolens	SAMN21168694	28.5	84.46
Achromobacter panacis	SAMN36085872	22.3	72.55
Achromobacter pestifer	SAMN11056505	50.4	92.76

(*Continued*)

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Table 2 (Continued).

Strain	Biosample	dDDH (%)	ANI (%)
Achromobacter piechaudii	SAMN00120564	28.5	84.27
Achromobacter pulmonis	SAMN20569911	28.4	84.29
Achromobacter ruhlandii	SAMN05715545	29.2	84.72
Achromobacter spanius	SAMEA3724092	28.3	84.10
Achromobacter veterisilvae	SAMEA4780387	34.2	87.28
Achromobacter xylosoxidans	SAMEA2517358	29.1	84.56
Achromobacter insolitus	SAMEA4384063	39	89.57
Achromobacter aegrifaciens	SAMEA6797398	**60.1**[a]	**94.76**[a]

Note: [a]The closest matches are highlighted in bold.

To understand the origins and clinical significance of the *Achromobacter* strain L3024hy, we conducted phylogenomic and comparative genomic analyses (Figure 1C). A phylogenetic tree showed that L3024hy is closely related to environmental strains from Chinese soil, indicating possible environmental sources for this pathogen. Despite minor genomic differences, L3024hy contained more antimicrobial resistance genes than its environmental counterparts (Table S1). Both strains shared the virulence gene, *brkB*, the few virulence factors identified are probably due to databases that may not fully cover the specialized or unknown virulence mechanisms of *Achromobacter* species. This suggests that clinical settings

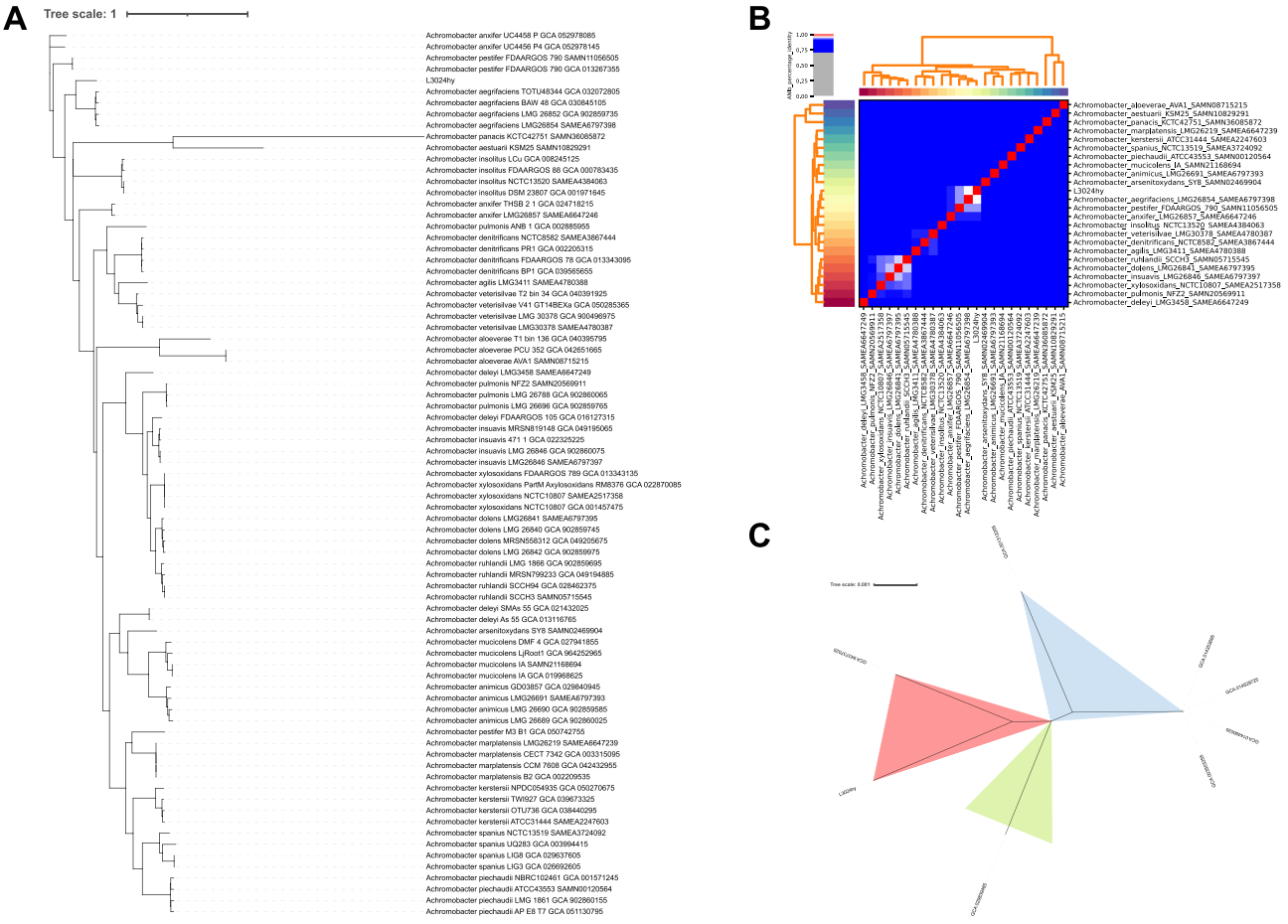

Figure 1 (**A**) Maximum likelihood phylogenomic tree based on the concatenated nucleotide sequence of core genes of strain L3024hy and type strains of the genus *Achromobacter*. (**B**) Average nucleotide identity analysis of strain L3024hy and type strains of the genus *Achromobacter*. (**C**) Maximum-likelihood phylogenetic tree based on core-genome SNPs of Novel *Achromobacter* strains.

drive resistance gene acquisition, making L3024hy a threat to multidrug resistance. The close relationship between clinical and environmental strains highlights the need for monitoring zoonotic transmission and antibiotic resistance.

In summary, extensive phylogenomic analysis utilizing Average Nucleotide Identity and digital DNA-DNA Hybridization values substantiated that strain L3024hy constitutes a distinct and novel species within the genus *Achromobacter*. The pronounced genotypic divergence, along with its unique genomic characteristics, justifies its taxonomic distinction from other recognized species. This discovery underscores the emergence of clinically significant *Achromobacter* lineages and emphasizes the critical role of integrating genomic taxonomy into pathogen surveillance systems to enhance monitoring and response strategies against the evolving threats of antimicrobial resistance.

Data Sharing Statement

This Whole Genome Shotgun BioProject for L3024hy has been deposited in GenBank under accession number PRJNA1227242.

Ethics Approval and Consent to Participate

This study was retrospective in nature, so informed consent was waived by the Clinical Research Ethics Committee of The First Affiliated Hospital, Zhejiang University School of Medicine. The patient treatment information has been de-identified and is in compliance with the Helsinki Declaration. All experiments strictly followed relevant guidelines and regulations, and the ethical protocol was approved by the Clinical Research Ethics Committee of The First Affiliated Hospital, Zhejiang University School of Medicine [No. 2018-752].

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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