

A Rare Case of *Gulosibacter massiliensis* Complicating Peritoneal Carcinomatosis

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Background: *Gulosibacter massiliensis*, a Gram-positive, strictly aerobic, and peroxidase-positive bacterium with high motility, was first isolated from a bloodstream infection in 2022. At present, clinical reports on *G. massiliensis* are limited, with only one case of skin and soft tissue infection reported, and its pathogenicity and infectivity are still unclear.

Case Presentation: A 74-year-old male patient with extensive peritoneal carcinomatosis with ascites infected by *G. massiliensis*. The patient was admitted to the hospital due to confusion of consciousness. After B-ultrasound, ascites cytology, laboratory testing and multi-department joint diagnosis, it was found that he had malignant ascites, *G. massiliensis* infection, hepatic encephalopathy, peritoneal secondary malignant tumor and other diseases. During the hospitalization, due to the patient's critical condition. The patient was deemed unfit for curative treatment and was managed with best supportive care. Ultimately, respecting the family's wishes, the patient left hospital and went home to receive palliative care. During the laboratory test, we used traditional biochemical reactions and matrix-assisted laser desorption ionization time-of-flight mass spectrometry failed to accurately identify the strain. Finally, the bacteria were finally identified as *G. massiliensis* by 16S rRNA gene sequencing and NCBI database alignment, and the drug susceptibility results of the bacteria were determined according to the evolutionary relationship and CLSI M45.

Conclusion: This study is the first report that *G. massiliensis* can cause ascites infection as an opportunistic pathogen in immunocompromised patients. It provides a reference for clinical laboratories to identify this rare pathogen and develops anti-infection programs and also points out the research direction for analyzing its pathogenic mechanism.

Keywords: *Gulosibacter massiliensis*, clinical report, microbial identification, ascites, *Gulosibacter* sp.

Introduction

Pathogenic bacteria pose a considerable threat to global public health and patient well-being.¹ As of 2021, 1,513 bacterial pathogens affecting humans have been identified, with 197 new species identified between 2011 and 2020, highlighting a notable increase in the diversity of bacterial species responsible for human infections worldwide.² *Gulosibacter massiliensis*, a novel bacterium, was identified by Yacouba et al in 2022. This bacterium belongs to the genus *Gulosibacter* of the family *Microbacteriaceae*. The species name is derived from the Latin name for Marseille, France, where the type strain was first isolated. *G. massiliensis* is a high G+C, Gram-positive, motile, non-spore-forming, rod-shaped, aerobic organism that is catalase-positive and oxidase-negative.^{3,4}

Biochemically, the organism exhibits positive reactions for esterase, esterase lipase, leucine arylamidase, and acid phosphatase, and demonstrates the ability to ferment rhamnose, citrate, and D-trehalose. Conversely, it yields negative results for enzymes such as alkaline phosphatase, lipase, and valine arylamidase.^{3,4} *G. massiliensis*, a novel bacterium, was first isolated from a blood specimen, suggesting its potential for human pathogenicity.^{3,4} However, the mechanisms underlying its pathogenicity and transmissibility remain unclear. To date, only one clinical case of infection has been documented, that is, in 2024, Li et al reported its identification in wound exudate from the fourth and fifth toes of a male patient.⁵ Further clinical investigations were warranted to elucidate the infectious and pathogenic characteristics of this bacterium. In our study, we isolated this strain from the ascitic fluid of a patient with extensive peritoneal carcinomatosis

and meticulously documented the entire diagnostic and treatment processes. The comprehensive procedure encompassing culture, isolation, and identification within the clinical laboratory was recorded in detail. In addition, we gathered and systematically organized pertinent data concerning bacteria within the *Gulosibacter* genus, thereby offering a valuable reference for other clinical laboratories for the detection of *G. massiliensis*.

Case Report

A 74-year-old man was admitted to the emergency department on December 6, 2024, with a one-day history of confusion and agitation. Subsequently, he was transferred to the neurology department with a diagnosis of “altered consciousness, Etiology Under Investigation”. The patient had a history of diagnoses at another hospital including colon cancer, secondary hepatocellular and intrahepatic bile duct malignancies, secondary malignant tumors of abdominal lymph nodes and peritoneum, severe malnutrition with cachexia, pulmonary embolism, grade III hypertension, and hepatic insufficiency. The patient received chemotherapy with the “mFOLFOX 6” regimen from September to November 2023, followed by “radical resection of right lower quadrant colon cancer + resection of liver metastases at segments S4 and S8 + hilar lymphadenectomy + resection of rectal mesenteric mass” along with associated chemotherapy in December 2023. Upon admission to our hospital, the physical examination revealed that the patient exhibited abdominal distension, dullness on percussion, non-palpable liver and spleen, poor overall condition, fair dietary intake, and absence of bowel movements for two days. Neurologically, the patient was somnolent, uncooperative during conversation and physical examination, and unable to perform the Romberg test successfully. Imaging studies, including abdominal and chest ultrasound and abdominal CT of external hospital, revealed a space-occupying lesion within the liver parenchyma, status post cholecystectomy, a hyperechoic area in the right hepatic lobe (hemangioma may be), splenomegaly and moderate to large volume of ascites (refer to Figure 1A and B).

Laboratory tests showed significant elevations in procalcitonin (PCT, ng/mL), white blood cell count (WBC, $\times 10^9/L$), absolute neutrophil count (NEUT#, $\times 10^9/L$), neutrophil percentage (NEUT%), interleukin-6 (IL-6, pg/mL), and C-reactive protein (CRP, mg/L). However, hemoglobin (HGB, g/L) showed a downward trend. (Figure 1C). Stool examination revealed the presence of mucus and blood, with a positive fecal occult blood test confirmed using the

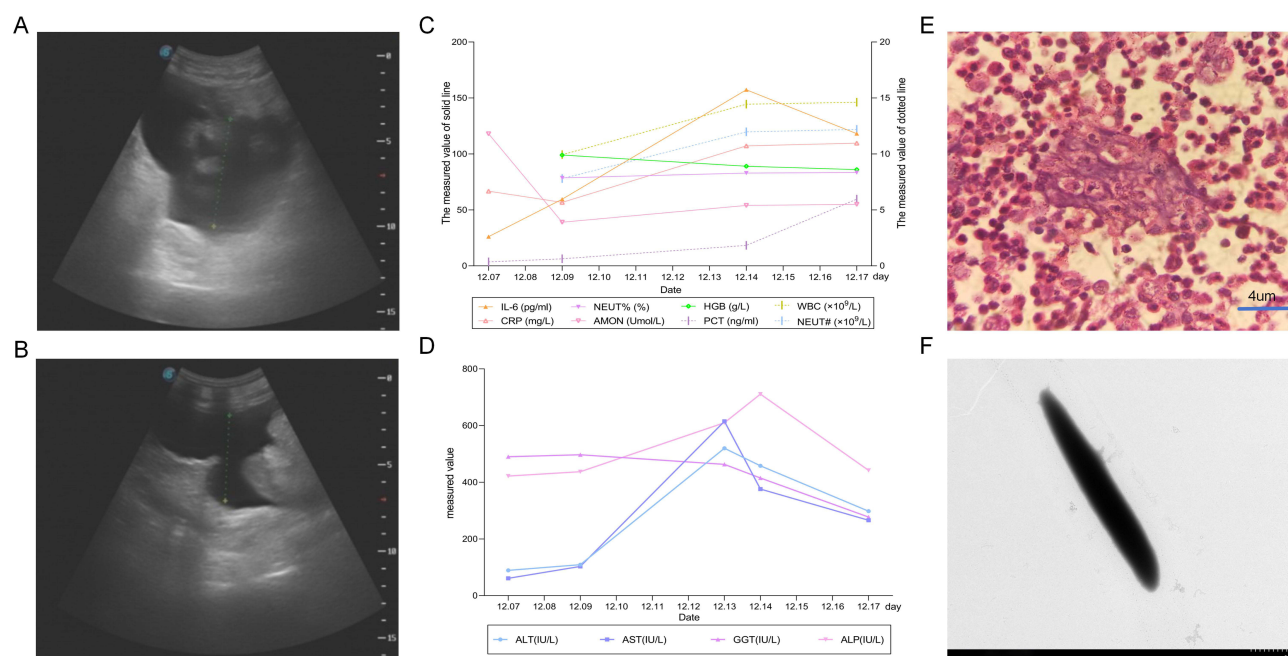


Figure 1 Auxiliary test results. (A and B) show abdominal ultrasound images; (C and D) show laboratory test results; (E) shows pathological image of ascitic fluid; (F) shows electron microscopic image of *G. massiliensis*.

Abbreviations: PCT, Procalcitonin; WBC, White Blood Cell Count; NEUT#, Neutrophil Absolute Count; IL-6, Interleukin-6; NEUT%, Neutrophil Percentage; HGB, Hemoglobin; CRP, C-Reactive Protein; AMON, Ammonia; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; GGT, Gamma-Glutamyl Transferase; ALP, Alkaline Phosphatase.

monoclonal antibody method. Given the presence of cough, sputum, and ascites, the possibility of pulmonary and abdominal infections was considered in the differential diagnosis. Cranial computed tomography (CT) revealed lacunar lesions in the bilateral basal ganglia and semioval center. These findings, in conjunction with cranial magnetic resonance imaging (MRI) and diffusion-weighted imaging (DWI), which indicated old lacunar cerebral infarctions and ischemic injury foci, as well as significantly elevated blood ammonia levels and transaminases (refer to [Figure 1C](#) and [D](#)), led to the diagnosis of hepatic encephalopathy as the underlying cause of the patient's confusion. The other diagnoses were consistent with those made at the external hospital. After cefoperazone/sulbactam (1.5 g, q12h) anti-infection, acidified intestinal tract (Enema with NS 500mL + Acetic Acid 10 mL, bid), L-aspartate ornithine ammonia reduction, nutritional support, maintenance of defecation regularity and symptomatic support treatment, the patient's consciousness level improved and was transferred to oncology department for treatment.

On the third day of hospitalization, paracentesis was performed at the junction of the lateral and middle thirds of the line connecting the umbilicus to the left anterior superior iliac spine, with the patient in a supine position. A drainage tube was subsequently inserted, allowing gradual removal of ascitic fluid in small, frequent volumes. During the hospital stay, 10.5 liters of ascitic fluid were extracted. During the paracentesis procedure, 10 mL of ascitic fluid was introduced into an aerobic culture bottle (Hapyear, China) and dispatched to the microbiology laboratory for bacterial culture and identification. On the fifth day of hospitalization, Pathological analysis of the ascitic fluid revealed numerous malignant tumor cells with morphological characteristics consistent with adenocarcinoma, confirming the presence of malignant ascites ([Figure 1E](#)). On the eighth day of hospitalization, *G. massiliensis* was isolated from the sample. The morphology of the electron microscope is shown in [Figure 1F](#). Following the results of the antimicrobial susceptibility testing, the antibiotic regimen was adjusted to Combination of meropenem and tigecycline.

In addition to admission diagnosis, hepatic encephalopathy, obstructive jaundice, malignant ascites, right cerebral artery stenosis, multiple old lacunar cerebral infarction, mild anemia, benign prostatic hyperplasia, splenomegaly and extrahepatic bile duct catheterization were also diagnosed. The patient's condition was complex, the prognosis was poor, and the condition was critical. Following discussions with the patient's family, who demonstrated understanding and provided consent, palliative symptomatic treatment was initiated. After the ninth day of hospitalization, Esomeprazole was administered for acid suppression and gastric protection to prevent stress-induced ulcer bleeding. Liver-protective therapy with glutathione and polyene phosphatidylcholine resulted in a significant reduction in transaminase levels. L-ornithine L-aspartate therapy was continued to reduce blood ammonia levels, which were stabilized at approximately 55 $\mu\text{mol/L}$ ([Figure 1C](#) and [D](#)). On the eleventh day of hospitalization, the attending physician observed subcutaneous hemorrhage and extensive ecchymoses on the patient's soles and left scapula. The coagulation parameters were as follows: prothrombin time (PT), 90.80 S; activated partial thromboplastin time (APTT), 142.30 S; and antithrombin III (AT-III), 39%. Plasma transfusions of 300 mL each were administered on days 11, 12, and 13 to replenish coagulation factors, with no adverse reactions observed post-transfusion. The patient's condition remained critical, characterized by multiple comorbidities and complications and deteriorating liver function. On the afternoon of December 19, 2024, family members chose to discharge so that patients could receive palliative care at home.

Microbiological Analysis

An aerobic culture bottle containing ascitic fluid provided by the clinical department was incubated in the BacT/Alert 3D 240 blood culture system (bioMérieux, France) at 35°C. A positive signal was detected after 36.72 h of incubation. Subsequently, 1–2 mL of culture broth was inoculated onto Columbia blood agar plates and McConkey agar plates (OXIOD, UK) and incubated at 35°C with 5% CO₂. After 24 h of incubation, small white colonies were observed on Columbia blood agar. Gram staining revealed the presence of Gram-positive rods (refer to [Figure 2 Ai](#) and [Aii](#)). After 48 h, the colonies appeared circular, beige, raised, and smooth, measuring 1–2 mm in diameter, and emitted an odor. Gram staining remained positive ([Figure 2 Bi](#) and [Bii](#)). Over a period of 5 days, the bacterium grew relatively slowly, with the colony color gradually deepening from beige. Gram staining consistently indicated positive results, and the bacteria tended to cluster ([Figure 2 Ai-Eii](#)).

Using a Hitachi TEM system at an acceleration voltage of 80.0kV and a magnification of 4.0k, the bacterial morphology was observed to be irregular and predominantly coccobacillary, as depicted in [Figure 1F](#). No bacterial growth was detected on

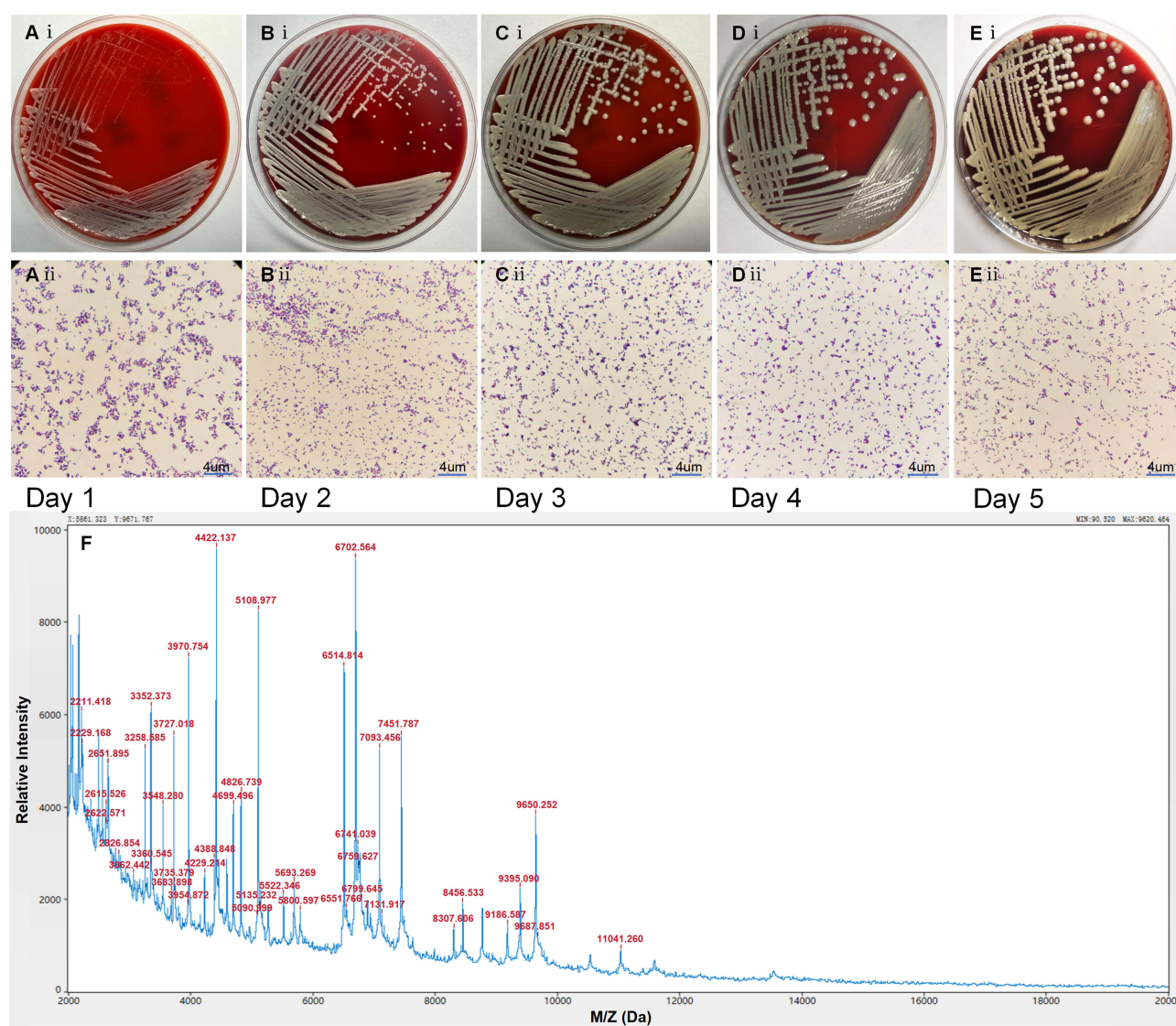


Figure 2 Colony morphology and Gram staining of *G. massiliensis* over five consecutive days of culture. **Ai–Ei**: Colony morphology on days 1–5; **Aii–Eii**: Gram stain images on days 1–5; **F**: Mass spectrometry image of *G. massiliensis*.

MacConkey agar or in anaerobic conditions. The bacterial samples were treated with 70% formic acid and an α -CHCA matrix solution (comprising 50% acetonitrile and 2.5% trifluoroacetic acid) and subsequently analyzed using an Autof ms1000 (AutoBio, China) matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS). Mass spectrometry analysis identified the bacterium as *Pseudoclavibacter faecalis*, with a score of 9.025. According to these criteria, confirmation was required for rare species, even with scores ≥ 6.0 , owing to insufficient database coverage, and sequencing was recommended. The mass spectrum, viewed using Auto Acquirer software, revealed characteristic peaks at 3970.754, 4422.137, 5108.997, and 6702.564, among others (Figure 2F).

Subsequent biochemical identification was conducted utilizing the Vitek-2 Compact system, employing specific biochemical cards tailored for Gram-negative bacilli, Gram-positive cocci, and *Corynebacterium*. Biochemical analysis of the gram-positive cocci and *Corynebacterium* cards revealed the bacterium's ability to metabolize D-sorbitol and D-mannose as sugar substrates and ferment mannose, maltose, and xylose. Enzymatic activity assays returned positive results for L-Pyrrolydonyl-Arylamidase, the Amylase, cyclodextrin hydrolysis, alkaline phosphatase, pullulan hydrolysis, phosphatidylinositol-specific phospholipase C, and Arginine dihydrolase. Additionally, the Novobiocin susceptibility test yielded a positive result. The specific biochemical reaction results are shown in Table 1. Despite these biochemical

Table 1 Biochemical Card Identification Results of Gram-Negative Bacilli, Gram-Positive Cocci, and Corynebacteria

Gram-Positive Cocci Biochemical Card			Gram-Negative Bacilli Biochemical Card			Corynebacterium Biochemical Card		
Abbreviation	Full name of biochemical reaction	Result	Abbreviation	Full name of biochemical reaction	Result	Abbreviation	Full name of biochemical reaction	Result
O129R	O/129 resistance test	-	APPA	L-Alanine-Phenylalanine-Proline Arylamidase	+	GLU(A)	Glucose fermentation	-
BGAL	β -Galactosidase test	-	AGLTp	Alpha-Glucosidase	-	MAL	Maltose fermentation	-
PHOS	Phosphatase test	-	BXYL	Beta-Xylosidase	-	MAN	Mannitol fermentation	-
dMAN	D-Mannose	+	SUCT	Succinate Utilization	-	SUC	Sucrose fermentation	-
SAC	L-Pyrrolydonyl-Arylamidase	+	CMT	Citrate (Simmons)	-	XYL	Xylose fermentation	+
AGLU	Alpha-Glucosidase	-	ADO	Adonitol	+	FRU	Fructose fermentation	-
PyrA	L-Pyrrolydonyl-Arylamidase	-	dGLU	D-Glucose	+	GLY	Glycerol fermentation	-
BGUR	β -Glucuronidase test	-	BAlap	Beta-Alanine Arylamidase pNA	+	GAL	Galactose fermentation	-
dSOR	D-Sorbitol	+	dTAG	D-Tagatose	+	RIB	Ribose fermentation	-
dMNE	Mannose fermentation (dextrose)	+	NAGA	N-Acetyl-Beta-Glucosaminidase	-	URE	Urease test	-
AMY	Amylase test	+	GGT	Gamma-Glutamyl Transferase	-	PYZ	Pyrazinamidase test	-
CDEX	Cyclodextrin hydrolysis test	+	ProA	L-Proline Arylamidase	+	NIT	Nitrate reduction test	-
dMAL	Maltose fermentation (dextrose)	+	dTRE	D-Trehalose	+	GLU	Glucose fermentation(anaerobic)	-
PUL	Pullulan hydrolysis test	+	AGAL	Alpha-Galactosidase	-	ESC	Esculin hydrolysis test	-
BGURr	β -Glucuronidase test (rapid)	-	IARL	I-Arylamidase	+	GEL	Gelatin liquefaction test	-
dXYL	Xylose fermentation (dextrose)	-	OFF	Fermentation of Glucose (Acid production)	+	ALP	Alkaline phosphatase test	+
BGAR	β -Galactosidase test	-	LIP	Lipase	-	SHT	Sorbitol fermentation test	-
dGAL	Galactose fermentation (dextrose)	-	CIT	Citrate	-	SIP	Sucrose inositol phosphatase test	-
NOVO	Novobiocin susceptibility test	+	GGAA	Glu-Gly-Arg-Arylamidase	-			
PIPLC	Phosphatidylinositol-specific phospholipase C test	+	dCEL	D-Cellobiose	+			
ADHI	Arginine dihydrolase test (arginine decarboxylase)	+	BGLU	Beta-Glucosidase	-			
AMAN	Amygdalin fermentation test	-	PLE	Phospholipase	+			
dRIB	Ribose fermentation (dextrose)	-	MNT	Malonate	+			
NC6.5	Growth in 6.5% NaCl	-	GlyA	Glycine Arylamidase	-			
SAL	Salicin fermentation test	-	IMLTa	Malonate (I)	-			
AspA	Aspartate aminopeptidase test	-	dMAL	D-Maltose	+			
ILATk	L-Lactate alkalization test	+	TyrA	Tyrosine Arylamidase	+			
AGAL	α -Galactosidase test	-	5KG	5-Keto-Gluconate	-			
LeuA	Leucine arylamidase test	+	ODC	Ornithine Decarboxylase	-			
LAC	Lactose fermentation test	-	ELLM	Ellman (Test for ChE)	-			
dTRE	Trehalose fermentation (dextrose)	+	H2S	Hydrogen Sulfide Production	-			
POLYB	Poly- β -hydroxybutyrate accumulation test	-	ILATK	L-Lactate Alkalinization	+			
APPA	Alanine-phenylalanine-proline arylamidase test	-	LDC	Lysine Decarboxylase	-			
ProA	Proline arylamidase test	+	ILATa	Lactate (I)	-			
NAG	N-Acetyl- β -glucosaminidase test	+	BNAG	Beta-N-Acetyl-Galactosaminidase	-			
MBdG	Methyl- β -D-glucopyranoside fermentation test	+	IHISa	Histidine (I)	-			
ADH2s	Arginine dihydrolase test (arginine decarboxylase)	+						
BACI	Bacitracin susceptibility test	+						
OPTO	Optochin susceptibility test	-						
dRAF	Raffinose fermentation (dextrose)	+						
AlaA	Alanine arylamidase test	+						
TryA	Tyrosine arylamidase test	+						

Notes: “+” indicates a positive biochemical reaction, while “-” indicates a negative biochemical reaction.

analyses, the precise strain could not be identified in this study. Consequently, 16S rRNA gene sequencing was selected for definitive identification and was performed by RuiBio BioTech. The gene amplification process resulted in a single distinct target band. BLAST analysis of the sequencing results indicated that the highest sequence similarities were observed with *G. massiliensis* (NR179448.1) at 99.35% and *Gulosibacter macacae* (NR180318.1) at 97.26%. Based on an integrated assessment of the biochemical characteristics, 16S rRNA sequencing data, and pertinent historical information, the bacterium was conclusively identified as *G. massiliensis*.

The 16S rRNA sequencing data were submitted to NCBI-BLAST using the core nucleotide database (core_nt) for Standard Nucleotide BLAST analysis. Appropriate comparison results were selected to construct a phylogenetic tree in MEGA 11, employing the ClustalW algorithm for sequence alignment and the neighbor-joining method for tree construction (Figure 3A and B). The *G. massiliensis*-3268 strain was classified within the *Gulosibacter* genus and exhibited the closest relationship to *Gulosibacter faecalis* strain C2 (PP_930902.1), with a sequence homology of 99.71%. The sequence homology with other species within the same genus exceeded 95%. Genera closely related to *Gulosibacter* sp., such as *Pseudoclavibacter* sp. and *Zimmermannella* sp., are classified within the phylum *Actinobacteria*, class *Actinobacteria*, order *Micrococcales*, and family *Microbacteriaceae*, suggesting a relatively close phylogenetic relationship, as illustrated in Figure 3A. Furthermore, *G. massiliensis*-3268 exhibited phylogenetic affinities with various other bacteria, most notably with *Zimmermannella faecalis* strain IFO15706 (AB 012591.1), demonstrating a sequence homology of 99.06%, indicative of a close relationship. Other species phylogenetically proximate to *G. massiliensis*-3268 include *Klugiella xanthotipulae* strain 44C3 (NR_042891.1), as depicted in Figure 3B. The closest relative identified was *Pseudoclavibacter* sp. Consequently, in accordance with the Clinical and Laboratory Standards Institute (CLSI) M45, 3rd Edition, 2015, the standard for *coryneform* and other *corynebacteria* was employed for antimicrobial susceptibility testing. The broth dilution method was used to determine the Minimum Inhibitory Concentration (MIC), while the Kirby-Bauer (K-B) method was used to measure inhibition zones, albeit not for interpretative purposes. According to the Clinical and Laboratory Standards Institute (CLSI) M45 interpretative criteria, *G. massiliensis* is susceptible to daptomycin, doxycycline, gentamicin, linezolid, vancomycin, meropenem, and tetracycline (Table 2).

Discussion

The genus *Gulosibacter* is classified within the domain Bacteria, phylum *Actinobacteria*, class *Actinobacteria*, order *Micrococcales*, and family *Microbacteriaceae*. Members of this genus are gram-positive, strictly aerobic, non-spore-forming, typically presenting as irregular short rods. The predominant fatty acids identified in this genus are 12-methyl tetradecanoic acid (anteiso-C15:0), 14-methyl pentadecanoic acid (iso-C16:0), and 14-methyl hexadecanoic acid (anteiso-C17:0). The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, and an unidentified glycolipid, whereas the major whole-cell sugars were ribose and rhamnose. The primary menaquinone in the respiratory chain is MK-9, and the genomic DNA exhibits a G+C content ranging from approximately 60.0 to 70.0 mol%.⁶ The optimal growth conditions for this genus are a temperature of approximately 30°C and pH of 8.0. They exhibit positive results for catalase, pyrazinamidase, and various other enzyme activities while testing negative for urease.⁷ Recognized species within this genus include the type species *G. molinativorax* and seven additional species: *Gulosibacter bifidus*, *Gulosibacter chungangensis*, *G. faecalis*, *Gulosibacter hominis*, *G. macacae*, *G. massiliensis*, and *Gulosibacter sediminis*.⁵ These species have been isolated from diverse samples across different countries and possess distinct characteristics.

G. molinativorax was isolated in 2004 and 2011, respectively, and can mineralize the herbicide Molinate to decompose into ethanethiol and acepan-1-carboxylate. Due to its significant phylogenetic distinctions from the genera *Curtobacterium* and *Brevibacterium helvolum*, it was classified as a new genus and species.⁸ It is the only bacterium known to hydrolyze the sulfate ester bond of molinate.⁹ This bacteria relies on molinate hydrolase, a cobalt-dependent enzyme with a predicted molecular mass of 50.9 kDa. Structurally, it exists as a homotetramer and belongs to subfamily A of the amidohydrolase superfamily, utilizing cobalt as its sole catalytic center.^{10,11} In 2017, the microencapsulation of *G. molinativorax* ON4T using modified chitosan tripolyphosphate crosslinking demonstrated favorable physical properties and effective degradability towards malonate.¹² In 2022, a novel composite transposon (Tn6311) harboring the singular catabolic gene *molA* was identified on a newly discovered low-copy-number plasmid (pARLON1). This gene, located on a chromosome, is predicted to encode an enzyme involved in the cleavage of heterocyclic rings.¹³

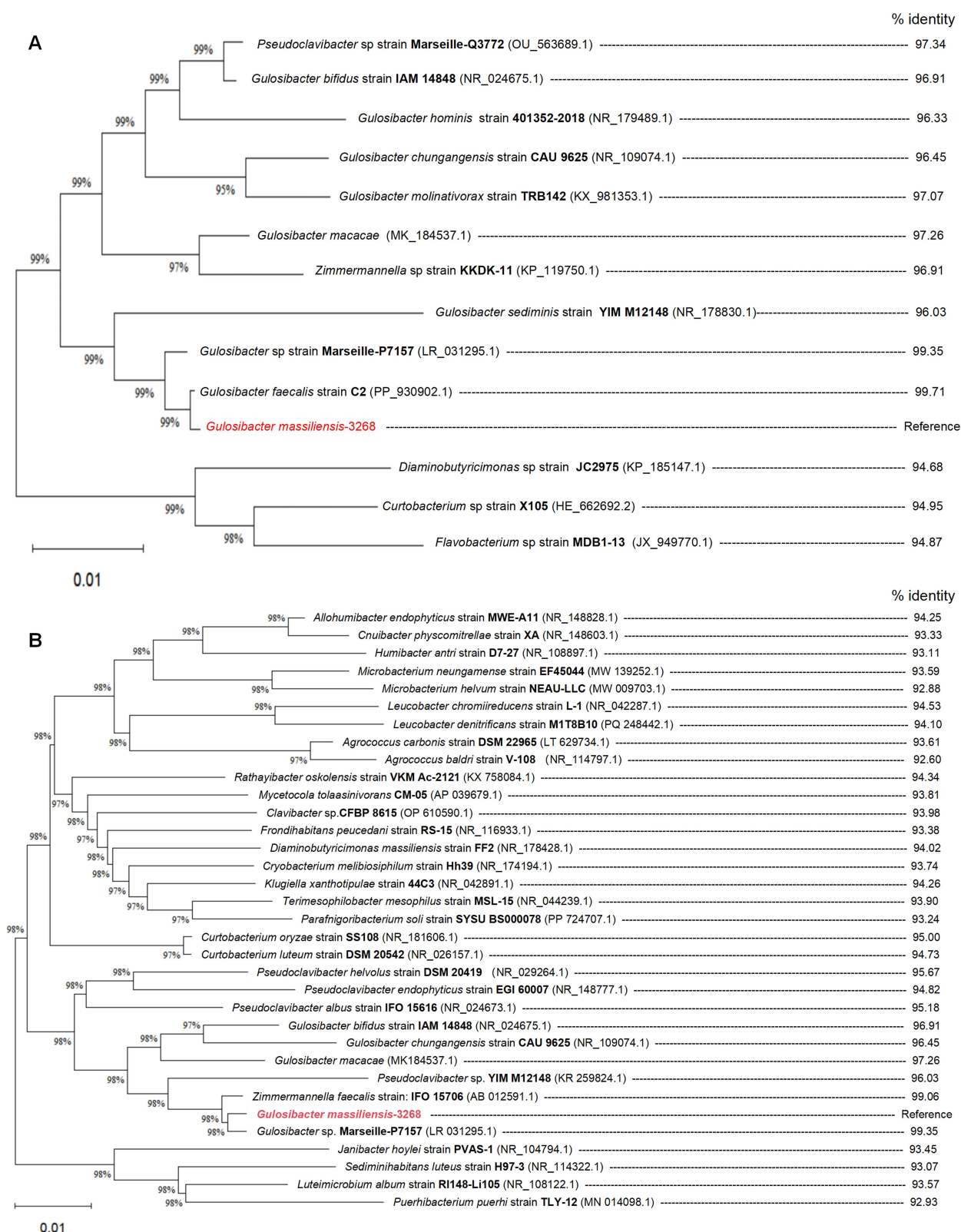


Figure 3 Phylogenetic tree based on the 16S rRNA gene sequence of *G. massiliensis*. **(A)** Phylogenetic tree relationship of *Gulosibacter* sp. and its related genera. **(B)** Phylogenetic relationship of *G. massiliensis* in Microbacteriaceae.

Notes: Reference represents the reference point of the phylogenetic tree; The red font represents the strain detected in this experiment; 3268 represents the strain number; coarsening indicates the specific numbered of bacterial strains; The contents in brackets indicate the accession number in NCBI; % identity represents the similarity ratio.

Table 2 Drug Susceptibility Test of *Gulosibacter massiliensis*

Abbreviation	Full Name of Antimicrobial Drugs	MIC	K-B Method	CLSI M45 Interpret
CLI	Clindamycin	1	27	I
PEN	Penicillin	1	18	I
FEP	Cefepime	>4	14	R
CTX	Cefotaxime	>4	12	R
CRO	Ceftriaxone	16	15	R
CIP	Ciprofloxacin	>4	6	R
E	Erythromycin	>8	10	R
RIF	Rifampin	>32	6	R
SXT	Trimethoprim/Sulfamethoxazole	4/76	24	R
DAP	Daptomycin	≤0.12	-	S
DOX	Doxycycline	≤0.12	-	S
GEN	Gentamicin	4	33	S
LNZ	Linezolid	≤1	44	S
MRP	Meropenem	≤0.12	44	S
TET	Tetracycline	≤0.5	-	S
VA	Vancomycin	0.5	38	S
AK	Amikacin	4	34	-
AMP	Ampicillin	1	25	-
ATM	Aztreonam	>64	6	-
FOX	Cefoxitin	>64	6	-
CAZ	Ceftazidime	>64	6	-
CXM	Cefuroxime	>64	6	-
C	Chloramphenicol	>32	6	-
IPM	Imipenem	≤0.12	45	-
LEV	Levofloxacin	4	15	-
TEC	Teicoplanin	≤0.5	31	-
TGC	Tigecycline	≤0.12	20	-
TOB	Tobramycin	4	20	-
AMC	Amoxicillin/Clavulanic acid	≤2	38	-
SCF	Cefoperazone/Sulbactam	≤8	40	-
MXF	Moxifloxacin	1	-	-
OFX	Ofloxacin	>8	-	-
OXA	Oxacillin	>4	-	-
ETP	Ertapenem	0.5	-	-
CFT	Ceftaroline	0.5	-	-
TCC	Ticarcillin/Clavulanic acid	16	-	-
CFP	Cefoperazone	-	40	-
PRL	Piperacillin	-	40	-
MH	Minocycline	-	39	-
TZP	Piperacillin/Tazobactam	-	42	-
SAM	Ampicillin/Sulbactam	-	26	-

Notes: MIC represents minimum inhibitory concentration, K-B Method represents disk diffusion method; R indicates resistant; I indicates intermediate; S indicates sensitive; “-” indicates none.

Additionally, in 2004, Lin et al isolated *G. faecalis* from bovine feces, whereas *G. bifidus* was isolated from soil, human blood, and wound samples. Initially, these two strains were classified as *Zimmermannella* species.¹⁴ In 2018, Nouioui et al reclassified *Actinobacteria* into the genus *Gulosibacter* species. Based on their genomic taxonomy. The transmissibility and pathogenicity of these two strains remain incompletely understood.¹⁵ *G. chungangensis* CAU 9625^T, isolated in 2012 from Yellow Sea sediments in South Korea, exhibits 97.8% 16S rRNA gene sequence similarity to model bacteria *G. molinivorax* ON4^T and possesses a comparable fatty acid composition.⁶ The strain *G. chungangensis* Gc4EO, isolated in 2021, produces a 4-ethylphenol oxidase. This enzyme shares 42% sequence identity with the vanillyl

alcohol oxidase (VAO) from *Corynebacterium glutamicum* and possesses the same 8 α -N3-histidine-linked FAD cofactor. Utilizing oxygen as the sole electron acceptor, it holds promise as a catalyst of choice for the specific dehydrogenation of phenolic compounds.¹⁶

Gulosibacter sp. YZ4, isolated from activated sludge in 2012, demonstrated heavy metal tolerance and phenol biodegradation. It can also be preserved via cell immobilization, rendering it suitable for purifying water samples with high phenol concentrations.¹⁷ In 2016, *Gulosibacter* sp. BS4, isolated from the activated sludge of a wastewater treatment facility at the Shchekino-Azot chemical plant in Russia, was confirmed to degrade ϵ -caprolactam and Nylon-6 oligomers.¹⁸ In 2019, *Gulosibacter* sp. BS38, isolated from soil samples contaminated with industrial waste ϵ -caprolactam, was capable of growing in a mineral medium with caprolactam as the sole carbon and energy source. It also exhibited the ability to degrade ϵ -caprolactam and its analogs and possessed 6-aminohexanoate-dimer hydrolase activity.^{19,20} In 2025, *Gulosibacter* species. ACHW.36C, isolated from an old cleaning sponge used for applying petroleum-based hoof oil to horses, demonstrated the ability to degrade toluene.²¹ Although these bacteria have not been formally taxonomically classified, they possess significant bioengineering potential and play important roles in biological processes.

In 2020, *G. macacae* was isolated from the feces of black langurs, and in 2021, *G. sediminis* was isolated from the marine sediments of the Indian Ocean. Both strains largely conform to the fundamental characteristics of *Gulosibacter* species, yet they do not exhibit distinctive features.^{7,22} Discovered in 2021, *G. hominis* was isolated from an ear infection and was part of the human skin microbiota and might play a role in opportunistic infections. Notably, it lacks acquired antimicrobial resistance determinants and virulence factors.²³ *G. massiliensis*, isolated from a blood specimen in 2022, is distinguished as the only highly motile strain within the *Gulosibacter* species and has been associated with wound infections and exudate formation.^{4,5} Overall, *Gulosibacter* species are widely distributed in nature and exhibit diverse biological functions, including the degradation of various substances, such as molinate, phenols, caprolactam, toluene, ϵ -caprolactam, and nylon-6 oligomers. Both *G. hominis* and *G. massiliensis* are implicated in opportunistic infections and have the potential to cause bacteremia, thus warranting increased scrutiny. The detailed characteristics of these strains are presented in Table 3.

The precise source of the *G. massiliensis* isolated from the ascitic fluid remains unconfirmed, we hypothesize a translocation from the gastrointestinal tract. This hypothesis is supported by the presence of concurrent gastrointestinal hemorrhage and widespread carcinomatosis peritonei. These conditions likely compromised the intestinal mucosal barrier, facilitating bacterial dissemination. Furthermore, the patient's immune competence was presumably impaired, either by the systemic immunosuppressive tumor microenvironment^{24,25} or as a sequela of oncologic therapies.²⁶ Given that *G. massiliensis* is characterized as the only highly motile strain within its genus, it possesses a distinct mechanistic advantage to invade the peritoneal cavity from the gut lumen. As a typical opportunistic pathogen within the *Gulosibacter* genus, *G. massiliensis* may present an elevated infection risk for oncological patients, potentially affecting their in-hospital survival outcomes.²⁷

Upon reviewing the entire clinical course, the patient was admitted with “one-day history of confusion and agitation”, and diagnosed with 18 medical conditions, including extensive peritoneal carcinomatosis, liver failure, moderate-to-large ascites with infection, multiple old lacunar cerebral infarctions, severe malnutrition with emaciation, and grade III hypertension etc. Given the critical condition, poor prognosis, and compromised immune status, only palliative care was feasible for alleviating suffering. Ultimately, respecting the family's wishes, the patient left hospital and went home to receive palliative care. This case expands the infection spectrum of *G. massiliensis* reported in the literature. However, this case has limitations such as poor prognosis, no ascites biochemistry, and failure to calculate the SAAG (Serum-Ascites Albumin Gradient) value. Given its atypical clinical manifestations, the exact systemic health effects of *G. massiliensis* remain unclear, and there is currently a lack of standardized treatment options for antibiotic selection and dosage. These uncertainties highlight the urgent need to further explore its pathogenesis and develop evidence-based treatment strategies. From a diagnostic perspective, clinical laboratories should consider 16S rRNA sequencing when rare coryneform organisms cannot be reliably identified by MALDI-TOF MS, and *G. massiliensis* should be recognized as a potential opportunistic pathogen in immunocompromised patients.

Table 3 Basic Information, Characteristics, and Journal Sources of Various Strains in *Gulosibacter* Sp

Year	Strain Name	Separate Source	Growth Conditions	Full Gene Length	GC % (Mol %)	Cell Wall	Type Lineage	Strain Characteristics	Journal Source (PMID/DOI)
2004	<i>Gulosibacter molinivorax</i>	Isolated from a mixed bacterial culture	10–41 °C	3379129 bp	65	D-ornithine	CCUG 49965 DSM 13485 JCM 13320 LMG 21909 ON4 ^T	Strict aerobic exercise, not acid-tolerant; positive for oxidase and catalase; capable of producing molinate hydrolase, able to mineralize the herbicide molinate; contains the major menaquinone MK-9.	15,143,025 36,109,598
2004	<i>Gulosibacter bifidus</i>	Isolated from human wounds, human blood, and soil	30 °C	2,127,204 bp	62	2,4-diaminobutyric acid and ornithine	CCUG 50209 DSM 17450 HHZL-00027 IAM 14848 JCM 12920 NBRC 103089 TISTR 1511	Gram-positive, strictly aerobic, does not form spores, and is non-motile. The diamino acid in peptidoglycan is D-ornithine; the main respiratory quinone is menaquinone MK-9; polar lipids include diphosphatidylglycerol, phosphatidylglycerol, and an unidentified glycolipid.	15388726 30,186,281
2004	<i>Gulosibacter faecalis</i>	Isolated from cow dung	30 °C pH 8.0	2,847,348 bp	67	2,4-diaminobutyric acid and ornithine	ATCC 13722 CCUG 37873 IAM 15030 JCM 12866 NBRC 15706 TISTR 1514 IFO 15706	Gram-positive, aerobic, non-spore-forming, irregularly shaped, short rod-shaped. The cell size is 0.3–0.4 × 0.5–1.1 micrometers. Produces oxidase and can oxidize D-glucose, D-fructose, D-mannose, rhamnose, inositol, and mannitol to produce acid.	15388726 30,186,281
2012	<i>Gulosibacter chungangensis</i>	Isolated from sediments in the Yellow Sea, Korea	37 °C pH 8.0 1% NaCl	3,238,579 bp	66.2	2,4-diaminobutyric acid and ornithine	CAU 9625 ^T KCTC 13959 ^T CCUG 60841 ^T	Gram-positive bacteria, strictly aerobic, non-spore-forming, with irregular short rod-shaped morphology. Major whole-cell sugars: ribose and glucose. Major polar lipids: diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid, and an unidentified lipid. Capable of producing 4-ethylphenol oxidase (Gc4EO).	21,685,251 34,523,783

2012	<i>Gulosibacter</i> sp. YZ4	Isolated from activated sludge	10–41°C pH 5–11	-	-	-	YZ4	Heavy Metal Tolerance: The culture can tolerate 60 mg/L of Cr, 320 mg/L of Pb, and 80 mg/L of Cd. Phenol Biodegradation: This strain secretes two enzymes, phenol hydroxylase and catechol 1,2-dioxygenase, and can degrade Cresol up to 750 mg/L within 26 hours under aerobic conditions. Immobilized cells can be stored long-term (up to 2 years) without a significant decline in degradation activity.	DOI: 10.1002/jctb.2689
2016	<i>Gulosibacter</i> sp. BS4	Isolated from activated sludge in the wastewater treatment facility of the Russian Shchekino-Azot Chemical Company	33°C pH 6.0	-	-	-	KU174112	Can simultaneously use caprolactam and low-molecular-weight linear nylon oligomers as the sole carbon and energy sources. Can degrade ϵ -caprolactam and nylon-6 oligomers.	DOI: 10.1134/S0026261716050052
2019	<i>Gulosibacter</i> sp. BS38	Isolated from soil samples contaminated by ϵ -caprolactam industrial waste	28°C pH 7.5	-	-	-	BS38	Can survive in mineral medium with caprolactam as the sole carbon and energy source. Able to degrade ϵ -caprolactam and related compounds, such as enantiomeric lactams and caprylolactam, and also possesses 6-aminocaproic acid dimer hydrolase. Can degrade epsilon-caprolactam and its homologs enantolactam and caprylolactam.	DOI: 10.17223/19,988,591/45/1,125,707,105
2020	<i>Gulosibacter macacae</i>	Isolated from the faeces of <i>Macaca mulatta</i>	30–37°C pH 8.0 1.0–3.0% (w/v) NaCl	2,821,440 bp	63	2,4-diaminobutyric acid and ornithine	YIM 102482–1 ^T DSM 102156 ^T CCTC AB 2016023 ^T	Strictly aerobic, non-motile, positive for catalase and pyrazinamidase, negative for urease and other enzymes. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, and an unidentified glycolipid.	32809927

(Continued)

Table 3 (Continued).

Year	Strain Name	Separate Source	Growth Conditions	Full Gene Length	GC % (Mol %)	Cell Wall	Type Lineage	Strain Characteristics	Journal Source (PMID/DOI)
2021	<i>Gulosibacter sediminis</i>	Isolated from Indian Ocean marine sediments	28°C pH 8.0 1.0–3.0% (w/v) NaCl	-	67.15	Rhamnose, ribose, glucose, and mannose	DSM 29154 KCTC 29660 YIM M12148	Gram-positive, catalase-positive, oxidase-negative, aerobic, non-motile. Major polar lipids: Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unknown phospholipid, and one unknown lipid.	34292143
2021	<i>Gulosibacter hominis</i>	Found in ear infections	30°C	2,340,181 bp	62.04	2,4-diaminobutyric acid and ornithine	401352-2018 ^T LMG 31778 ^T CCUG 74795 ^T	Members of the human skin microbiota that occasionally participate in opportunistic infections. The main polar lipids include diphosphatidylglycerol, phosphatidylglycerol, and an unidentified glycolipid.	34480670
2022	<i>Gulosibacter massiliensis</i>	Isolated from blood specimens and wound secretions	25–37°C 48h	2,075,628 bp	67.2	-	Marseille-P7157 ^T CECT 30048	Gram-positive bacteria, motile, non-spore-forming, rod-shaped, and aerobic. It grows slowly, can catalyze the catalase reaction, but cannot perform the oxidase reaction. The main fatty acid is 12-methyltetradecanoic acid. Opportunistic pathogen.	36240159 35,460,225 39,538,156
2025	<i>Gulosibacter</i> sp. ACHW.36C	The old sponge used to apply petroleum-based hoof oil is separated from the horseshoe.	30°C pH 8.0	2,748,452 bp	67.61	-	ACHW.36C	Gram-positive, irregular rod-shaped, aerobic and non-motile, capable of decomposing toluene; positive for the activities of catalase, pyrazinamidase, pyrrolidinone arylaminase, lysine decarboxylase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, cystine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase.	40444951

Note: “-” indicates none; GC% represents GC content.

Ethics Statement

Approval has been obtained from the Ethics Committee of the First Hospital of Kunming, with the ethical approval number: Research Ethics Review (Single) - 2026-030-01. Informed Consent and Publication Consent: Written informed consent and consent for publication in journals have been obtained from the legally authorized representative of the patient (the patient's daughter) for the detailed case information involved in this study, including clinical data and accompanying images. This study strictly follows all principles in the Declaration of Helsinki and the CARE guidelines and does not involve personal privacy or commercial interests.

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Disclosure

The authors assert that the study was conducted without any business or financial affiliations that could be construed as a potential conflict of interest.

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