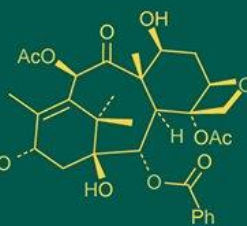
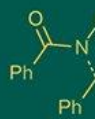


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Isolation and Functional Characterization of a Gut-Associated *Morganella morganii* from Wild-Caught *Metapenaeus dobsoni*

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Abstract

The gastrointestinal microbiota of wild aquatic organisms constitutes a significant reservoir of host-associated bacteria bearing functional characteristics pertinent to aquaculture. This research isolated and characterized a gut-associated bacterial strain (S9B43) from a healthy wild-caught pink shrimp (*Metapenaeus dobsoni*) employing phenotypic, biochemical, molecular, and functional methodologies. Conventional biochemical characterization provisionally identified the isolate as *Pseudomonas pseudoalcaligenes* (45.24% probability), whereas 16S rRNA gene sequencing definitively confirmed its identity as *Morganella morganii* (100% sequence identity). Isolate S9B43 demonstrated *in vitro* antagonistic activity against *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, *Edwardsiella tarda*, and *Streptococcus iniae*, and exhibited tolerance to a broad spectrum of environmental conditions, including pH 3-11, temperatures ranging from 4 °C to 45°C, 0-7% NaCl, and up to 3% bile salts. Nonetheless, the isolate also displayed α -haemolytic activity and resistance to antibiotics such as ampicillin, erythromycin, and oxytetracycline, indicating that further genomic characterization and comprehensive safety assessments are necessary prior to any practical application. The notable discrepancy between biochemical and molecular identification underscores the limitations inherent in traditional phenotypic methods for environmental bacterial identification. Overall, this study enhances existing knowledge regarding the cultivable gut microbiota of wild *M. dobsoni* and identifies *M. morganii* S9B43 as a biologically significant host-associated bacterium deserving of further ecological and genomic research.

Keywords: *Morganella morganii*, *Metapenaeus dobsoni*, gut microbiota, host-associated bacteria, probiotics, aquaculture

Introduction

Aquaculture represents one of the most rapidly expanding sectors within global food production. It is integral to securing food security, enhancing nutritional quality, and supporting the livelihoods of countless individuals. The industry has seen consistent growth over recent decades, with worldwide aquaculture output persistently rising to meet the escalating demand for aquatic food items ^[1, 2].

Notwithstanding this notable growth, disease outbreaks persist as a significant constraint on sustainable aquaculture production. Bacterial pathogens, especially species within the genera *Aeromonas*, *Vibrio*, *Edwardsiella*, *Streptococcus*, and *Pseudomonas*, are accountable for considerable economic losses due to heightened mortality rates, compromised growth, decreased feed efficiency, and deterioration of product quality in cultured fish and crustaceans ^[3-6].

For numerous decades, antibiotics have been extensively employed to prevent and treat bacterial infections within aquaculture. Nevertheless, the indiscriminate and prolonged administration of antimicrobial agents has facilitated the emergence and dissemination of antimicrobial-resistant bacteria, disturbed beneficial microbial communities, caused environmental contamination, and led to the accumulation of antibiotic residues in aquatic products. These issues collectively pose significant risks to animal health, environmental integrity, and public health ^[4, 7-9].

Consequently, there is a growing emphasis on the development of sustainable alternatives that improve host health while decreasing dependence on antimicrobial chemotherapy. Among the various biological strategies examined, the utilisation of host-associated beneficial microorganisms has become one of the most promising methods for enhancing disease resistance, preserving intestinal microbial homeostasis, and supporting the overall health of farmed aquatic animals [10-13].

Materials and Methods

Sample Collection and Processing

A healthy, wild-caught pink shrimp (*Metapenaeus dobsoni*) was collected from the Malpe fishing harbour, Udupi District, Karnataka, India (13°20'47.30" N, 74°42'3.93" E) in February 2024. The specimen was transported to the Aquatic Animal Health Management Laboratory, College of Fisheries, Mangaluru, under chilled conditions (4±1°C). Total length, body weight, and gut weight were recorded before aseptic dissection. The gastrointestinal tract was excised, homogenised in sterile phosphate buffered saline (PBS; pH 7.2) using a Stomacher® 400 (Seward Ltd., UK), and processed immediately for bacterial isolation [14-17].

Isolation and Purification of the Bacterial Isolate

Gut homogenates were enriched in alkaline peptone water (APW) at 30±2°C for 18 hours with agitation at 120 rpm. They were then serially diluted in sterile physiological saline and plated onto HiCrome® Bacillus Agar (HiMedia Laboratories, India). After incubation at 30±2°C for 18 to 48 hours, morphologically distinct colonies were purified by repeated streaking on Luria Bertani (LB) agar. A representative isolate, designated S9B43, was selected for further characterization based on colony morphology.

Phenotypic Characterization

Colony morphology, Gram reaction, cellular morphology, and motility were assessed employing standard microbiological procedures subsequent to incubation on LB agar at 30±2°C for a duration of 24 hours. Colony characteristics were documented according to size, pigmentation, margin, elevation, surface texture, and opacity. Furthermore, Gram staining and the hanging-drop method were utilised to determine cellular morphology and motility, respectively [16, 21, 22].

Biochemical Characterization

The isolate was characterised utilising conventional biochemical assays, including catalase, oxidase, indole production, methyl red, Voges-Proskauer, citrate utilisation, urease, nitrate reduction, hydrogen sulphide production, oxidation-fermentation, ONPG, phenylalanine deaminase, gelatin hydrolysis, starch hydrolysis, bile esculin hydrolysis, and triple sugar iron reactions. Carbohydrate fermentation and amino acid decarboxylase activities were assessed in accordance with standard microbiological protocols. The biochemical profile was analysed using ABIS online and contrasted with the descriptions provided in Bergey's Manual of Systematics of Archaea and Bacteria for initial taxonomic identification [21-24].

Molecular Identification

Genomic DNA was extracted using the CTAB method. The

16S rRNA gene was amplified with the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTTGTACGACTT-3'). PCR products were verified by electrophoresis on a 1.5% agarose gel, purified, and bidirectionally sequenced by Sanger (Barcode Biosciences Pvt. Ltd., Bengaluru, India). The consensus sequence was analysed using BLASTn against the NCBI nucleotide database, and phylogenetic analysis was performed in MEGA12 using the neighbour-joining algorithm with 1,000 bootstrap replications [25-30].

Functional Characterization

Antibacterial Activity

The antagonistic activity of isolate S9B43 against *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, *Edwardsiella tarda*, and *Streptococcus iniae* was assessed using the agar spot assay. Cell-free supernatant (CFS) and crude cell lysate (CL), prepared from overnight cultures, were evaluated separately through the spot-on-lawn method. Antibacterial activity was determined by observing inhibition zones surrounding the inoculation sites.

Antibiotic Susceptibility

Antibiotic susceptibility was assessed utilising the Kirby-Bauer disc diffusion technique on Mueller Hinton agar, employing commercial antibiotic discs that contained ampicillin (25 µg), erythromycin (15 µg), chloramphenicol (25 µg), enrofloxacin (10 µg), oxytetracycline (30 µg), and sulfonamide (300 µg). The isolate was classified as susceptible, intermediate, or resistant in accordance with the guidelines set forth by CLSI [34, 35].

Haemolytic Activity

Haemolytic activity was evaluated using 5% sheep blood agar after incubation at 30±2°C for a duration of 24-48 hours. Haemolytic reactions were documented as α-, β-, or γ-hemolysis according to the appearance of haemolytic zones surrounding the colonies [36, 37].

Tolerance to Environmental Conditions

The resilience of isolate S9B43 to various environmental conditions was evaluated through cultivation in LB broth across a spectrum of parameters, including temperatures (0-80°C), pH levels [1-11], NaCl concentrations (0-11%, w/v), and ox gall bile salt concentrations (0-3%, w/v). Following incubation at 30±2°C for 24 hours, bacterial proliferation was quantified by measuring the optical density at 600 nm (OD₆₀₀) [10, 38, 39].

Chemical Tolerance

Resistance to selected chemical agents, including phenol, ethanol, hydrogen peroxide, formalin, acetone, and sodium hypochlorite, was assessed utilising the disc diffusion method on Mueller Hinton agar. Following incubation at 30±2°C for a duration of 24 hours, the inhibition zones were measured to determine the susceptibility of the isolate to each chemical agent [35, 40].

Statistical Analysis

All experiments were conducted in duplicate, and the results are presented as the mean ± Standard deviation (SD). Descriptive statistics were calculated using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA) [41].

Results

Sample Characteristics and Bacterial Isolation

The wild-caught pink shrimp specimen (S9) (Figure 1a) was collected from Malpe, Udupi, Karnataka (Figure 1b) utilised in this study measured 13.50 cm in total length and had a weight of 20.60 g, with a gastrointestinal content weight of 0.704 g, accounting for approximately 3.4% of the total

body weight (Table 1). Selective cultivation of the intestinal homogenate derived from the wild-caught pink shrimp produced several bacterial colonies with distinct morphological characteristics. Following repeated streaking on Luria Bertani agar, a pure culture designated S9B43 was selected for comprehensive characterisation, based on its unique morphological features.

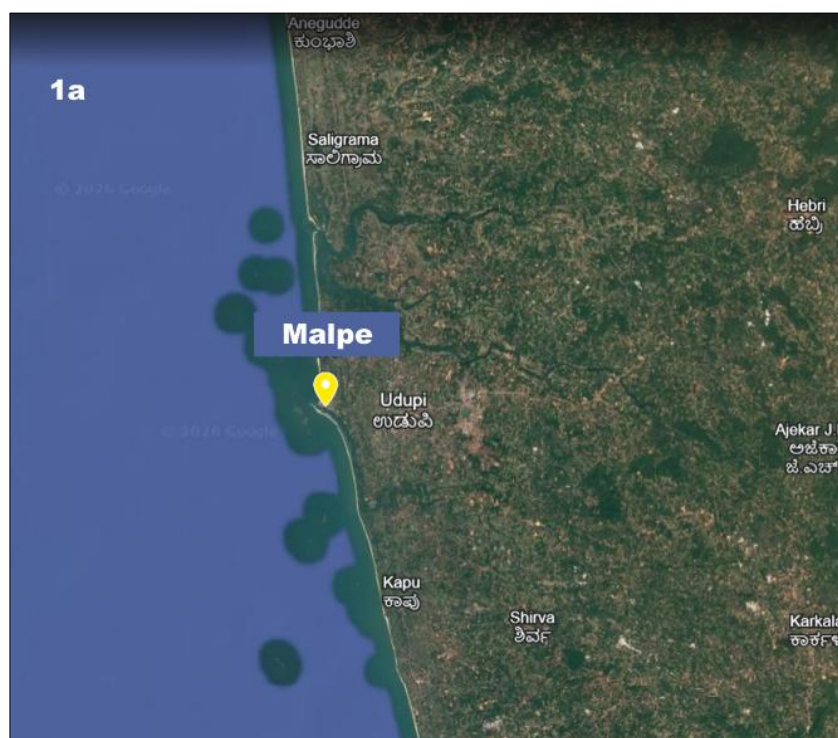


Fig 1a: Geographic location of the sample site

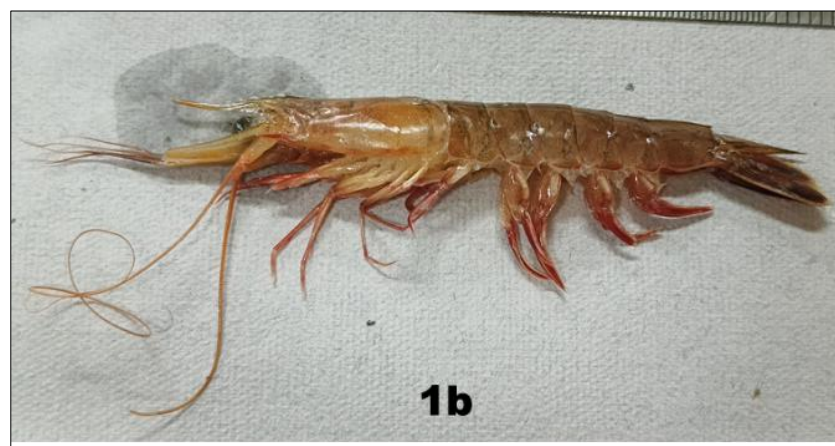


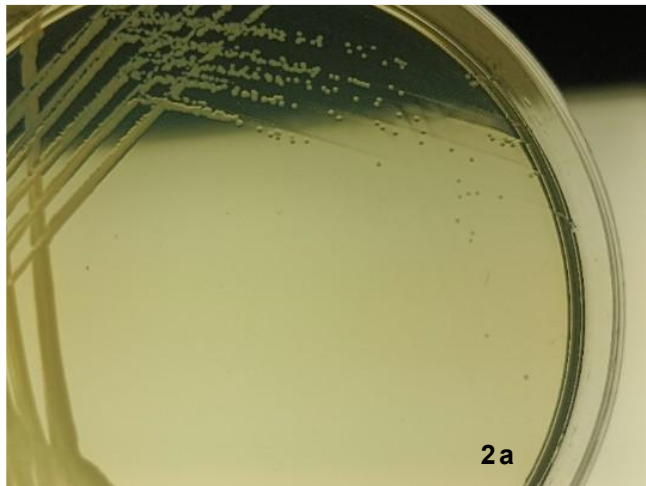
Fig 1b: *Metapenaeus dobsoni* (pink shrimp) collected in the study

Table 1: Sample characteristics of *Metapenaeus dobsoni* (S9) and the bacterial isolate

Parameter	Value
Date	11.02.2024
Place	Malpe, Udupi, Karnataka
GPS Coordinates	Lat: 13° 20' 47.30" N, Long: 074° 42' 3.93" E
Sample ID	S9
Species	<i>Metapenaeus dobsoni</i> (Pink shrimp)
Total length (cm)	13.50
Body weight (g)	20.60
Gut weight (g)	0.704
Gut weight (% of body weight)	3.42%
Isolate code	S9B43

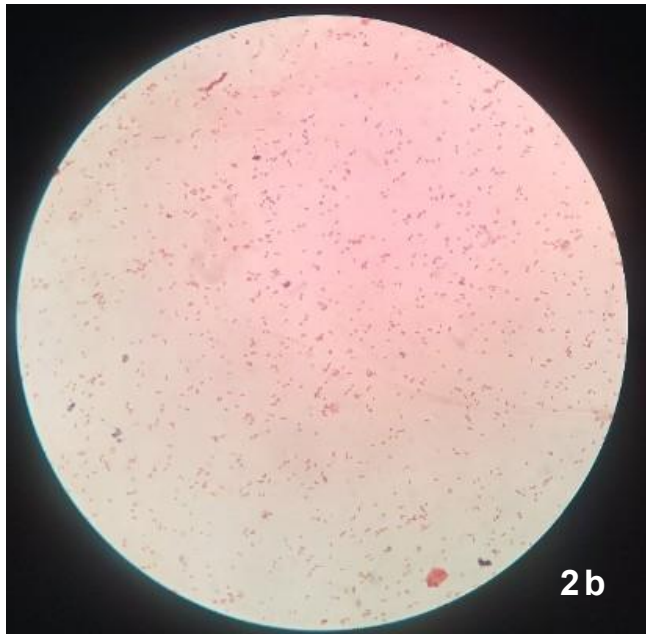
Morphological and Biochemical Identification

The isolate S9B43 yielded circular, cream-coloured colonies exhibiting entire margins, convex elevation, and a smooth, opaque surface after incubation on Luria Bertani agar (Figure 2a). Microscopic analysis demonstrated Gram-negative, rod-shaped, motile cells (Figure 2b). These phenotypic traits served as the foundation for subsequent biochemical and molecular assessments. Furthermore, the isolate was identified as non-motile, as determined via hanging drop methodology.



Small, circular, cream-coloured with a raised, smooth surface and an opaque

Fig 2a: Colony morphology of S9B43 on LBA plate



Cellular morphology displaying a negative Gram reaction, bacilli and monocellular arrangement

Fig 2a: Microscopic view of Gram staining of S9B43

A comprehensive biochemical characterization revealed a complex metabolic profile (Table 2). The isolate tested positive for indole production and the methyl red test, whereas it tested negative for Voges-Proskauer and citrate utilization. The triple Sugar Iron (TSI) agar test indicated an acid/acid reaction with gas production (A/A G), signifying fermentation accompanied by gas formation. The oxidation-fermentation (O/F) test yielded positive results for both

oxidative and fermentative metabolic pathways (+/+), demonstrating metabolic versatility. The isolate exhibited positive reactions for nitrate reduction, hydrogen sulfide production, catalase, oxidase, and phenylalanine deaminase activities; however, it was negative for urease, ONPG, gelatinase, starch hydrolysis, coagulase, and bile esculin tests. Carbohydrate fermentation analysis revealed selective sugar utilisation; the isolate fermented fructose, galactose, glucose, mannose, and ribose with gas production, whereas it did not ferment adonitol, arabinose, dulcitol, erythritol, inositol, lactose, maltose, mannitol, raffinose, sorbitol, or xylose.

Based on the comprehensive biochemical profile, the isolate was identified as belonging to the phylum Pseudomonadota (Proteobacteria), family Pseudomonadaceae, genus *Pseudomonas*. Species-level identification indicated *Pseudomonas pseudoalcaligenes* with the highest probability (45.24%), followed by *Pseudomonas stutzeri* (27.96%), *Xanthomonas maltophilia*/*Pseudomonas*-like species (22.93%), and *Pseudomonas vesicularis* (3.33%). The overall confidence level for the identification was moderate (Table 3).

Table 2: Biochemical characterisation of isolate S9B43.

Test	Results	Test	Results
Indole production	+	Carbohydrate fermentation	
Methyl Red (MR)	+	Adonitol	-
Voges-Proskauer (VP)	-	Arabinose	-
Citrate utilization	-	Dulcitol	-
Triple Sugar Iron (TSI)	A/A G (Acid/Acid with Gas)	Erythritol	-
		Fructose	+(with gas)
Oxidation-Fermentation (O/F)	+/+	Galactose	+(with gas)
Nitrate reduction	+	Glucose	+(with gas)
H ₂ S production	+	Inositol	-
Catalase	+	Lactose	-
Oxidase	+	Maltose	-
Urease	-	Mannitol	-
ONPG	-	Mannose	+(with gas)
Gelatinase	-	Raffinose	-
Starch hydrolysis	-	Ribose	+(with gas)
Bile esculin	-	Sorbitol	-
Phenylalanine deaminase	+	Xylose	-

Table 3: Taxonomic identification and species identification probabilities of S9B43.

Phylum	Pseudomonadota (Proteobacteria)
Family	Pseudomonadaceae
Genus	Pseudomonas
Species probability	
<i>Pseudomonas pseudoalcaligenes</i>	45.24% (highest)
<i>Pseudomonas stutzeri</i>	27.96%
<i>Xanthomonas maltophilia</i> / <i>Pseudomonas</i> -like spp.	22.93%
<i>Pseudomonas vesicularis</i>	3.33%
Identification confidence level	Moderate

Molecular Identification

Molecular identification by 16S rRNA gene sequencing confirmed isolate S9B43 as *Morganella morganii*, belonging to the phylum Pseudomonadota (Proteobacteria), family Morganellaceae, order Enterobacterales, and class

Gammaproteobacteria. BLAST analysis against the NCBI GenBank database yielded 100% nucleotide identity with the *M. morganii* PX313556.1 reference sequence. The aligned sequence was submitted to the NCBI database as *Morganella morganii*, and accession number PZ769661 was obtained. The nucleotide sequence was subsequently aligned with closely related reference sequences, and phylogenetic analysis confirmed clustering of the isolate within the *Morganella morganii* clade with high bootstrap support,

thereby validating its taxonomic identity (Figure 3). This result superseded the phenotypic identification, which had tentatively assigned the isolate to *Pseudomonas pseudoalcaligenes* (45.24% probability, moderate confidence). The discordance between phenotypic and molecular identification underscores the limitations of biochemical profiling systems for environmental isolates and highlights the indispensable role of molecular techniques in accurate taxonomic resolution.

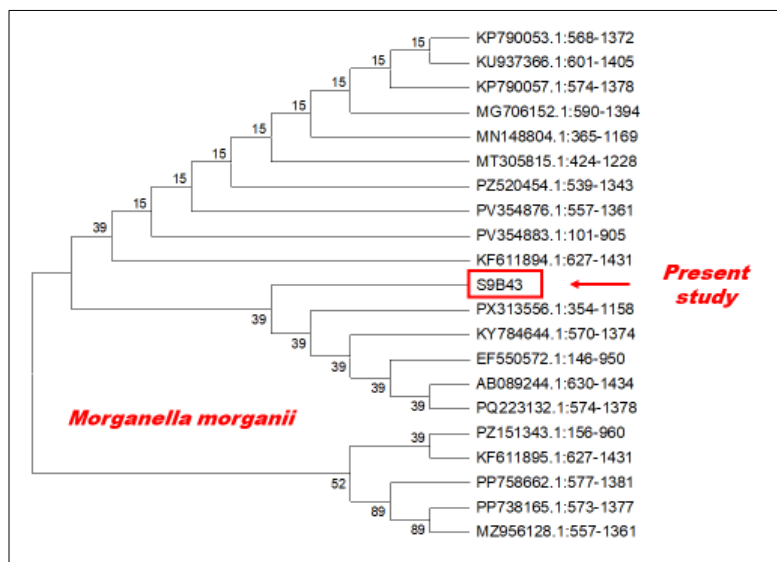


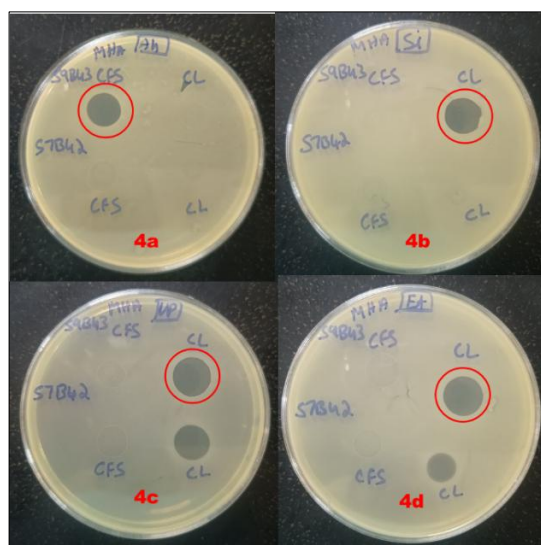
Fig 3: Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of *Morganella morganii* isolate S9B43 with closely related reference strains

Antibacterial Activity

The antagonistic activity of isolate S9B43 against aquaculture pathogens exhibited a pattern dependent on the pathogen and fraction (Figure 4). Cell lysate (CL) demonstrated inhibitory activity against *Vibrio parahaemolyticus* (VP), *Edwardsiella tarda* (ET), and *Streptococcus iniae* (SI). Conversely, CFS displayed inhibitory activity against *Aeromonas hydrophila*. This differential activity indicates the presence of distinct inhibitory substances in the cell-associated and extracellular fractions.

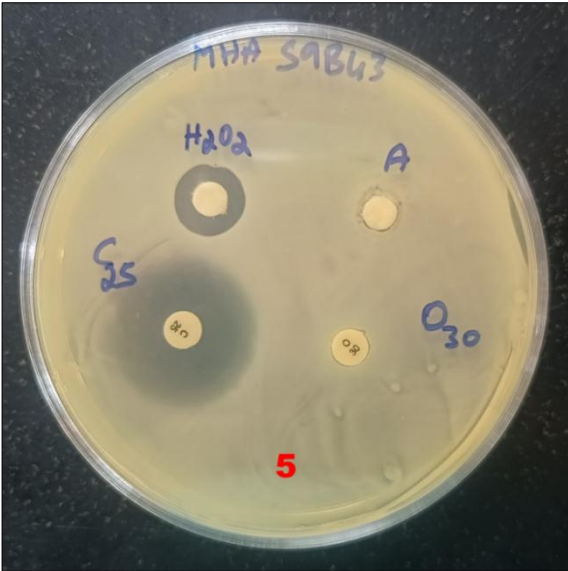
Antibiotic Susceptibility Testing

Antibiotic susceptibility testing disclosed a hybrid resistance profile (Table 4; Figure 5). The isolate exhibited resistance to ampicillin (AMP25: zone of inhibition = 10.000±1.414 mm) and erythromycin (E15: 9.000±1.414 mm), and was entirely resistant to oxytetracycline (OTC30: absence of inhibition zone). An intermediate level of susceptibility was detected for chloramphenicol (C25: 19.000±1.414 mm) and sulfonamide (SF300: 16.000±1.414 mm). Conversely, the isolate demonstrated susceptibility to enrofloxacin (EX10: 22.000±1.414 mm).



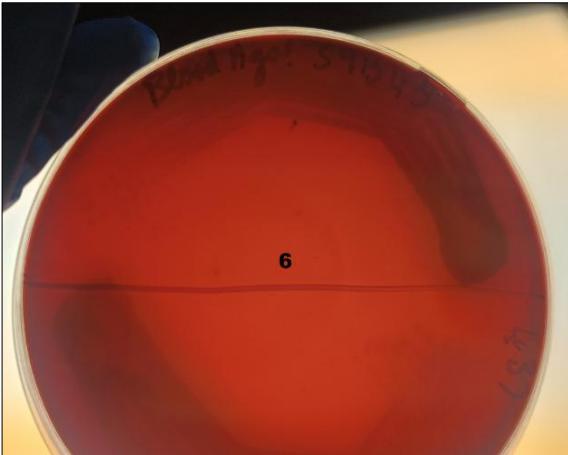
(4a) *Aeromonas hydrophila*, (4b) *Streptococcus iniae*, (4c) *Vibrio parahaemolyticus*, and (4d) *Edwardsiella tarda*. CFS: cell-free supernatant; CL: crude cell lysate. The CFS inhibited *A. hydrophila*, whereas the CL inhibited *S. iniae*, *V. parahaemolyticus*, and *E. tarda*

Fig 4: Antibacterial activity of *Morganella morganii* S9B43 against selected aquaculture pathogens determined by the spot-on-lawn assay



H₂O₂: hydrogen peroxide; A: acetone; C₂₅ chloramphenicol; O₃₀ oxytetracycline. Clear zones surrounding the discs indicate susceptibility to the respective chemical agents

Fig 5: Susceptibility of *Morganella morganii* S9B43 to selected chemical agents and antibiotics determined by the disc diffusion assay



The isolate exhibited α -haemolysis, evidenced by partial haemolysis surrounding the bacterial growth

Fig 6: Haemolytic activity of *Morganella morganii* S9B43 on 5% sheep blood agar

Hemolytic Activity Assessment

Assessment of hemolytic activity on sheep blood agar demonstrated that isolate S9B43 exhibited alpha (α) hemolysis (Figure 6). characterised by partial lysis of erythrocytes and a greenish discoloration surrounding the colonies. This pattern of hemolysis is regarded as more favourable from a safety standpoint in comparison to beta-hemolytic bacteria.

Chemical Tolerance: Chemical resistance testing revealed variable susceptibility to different chemical agents (Table 4; Figure 7). The isolate showed complete resistance to ethanol and acetone, with no zones of inhibition. However, it was susceptible to phenol and formalin, both producing identical zones of inhibition of 31.000 ± 1.414 mm. Moderate susceptibility was observed against hydrogen peroxide (zone of inhibition: 11.000 ± 0.707 mm) and sodium hypochlorite (zone of inhibition: 8.500 ± 1.414 mm).

Table 4: Antibiotic and chemical susceptibility of isolate S9B43

Parameter	Condition / Agent	Result (ZOI in mm)
Antibiotic	Ampicillin AMP25 (25 μ g)	10.000 $\pm$ 1.414 (Resistant)
	Erythromycin E15 (15 μ g)	9.000 $\pm$ 1.414 (Resistant)
	Chloramphenicol C25 (25 μ g)	19.000 $\pm$ 1.414 (Intermediate)
	Enrofloxacin EX10 (10 μ g)	22.000 $\pm$ 1.414 (Susceptible)
	Oxytetracycline OTC30 (30 μ g)	Resistant (no zone)
	Sulfonamide Sf300 (300 μ g)	16.000 $\pm$ 1.414 (Intermediate)
Chemical agent	Phenol (P)	31.000 $\pm$ 1.414 (Sensitive)
	Ethanol (Et)	Resistant (no zone)
	Hydrogen peroxide (H ₂ O ₂)	11.000 $\pm$ 1.414 (Moderate)
	Formalin (F)	31.000 $\pm$ 1.414 (Sensitive)
	Acetone (A)	Resistant (no zone)
	Sodium hypochlorite (SH)	8.500 $\pm$ 0.707 (Moderate)

Tolerance to different environmental conditions

The characterisation of the probiotic properties of isolate S9B43 demonstrated distinct tolerance to environmental stresses (Table 5). An assessment of temperature tolerance indicated that the isolate exhibited optimal growth at 30°C ($OD_{600} = 0.140 \pm 0.138$). Moderate growth was observed at 20°C ($OD_{600} = 0.110 \pm 0.108$) and 15°C ($OD_{600} = 0.100 \pm 0.098$). Growth was significantly reduced at 37°C ($OD_{600} = 0.090 \pm 0.092$) and 4°C ($OD_{600} = 0.090 \pm 0.092$), with minimal but discernible growth at 45°C ($OD_{600} = 0.040 \pm 0.038$) and 80°C ($OD_{600} = 0.020 \pm 0.018$). No growth was detected at 0°C.

pH tolerance experiments demonstrated that the isolate exhibited optimal growth at neutral pH 7 ($OD_{600} = 0.130 \pm 0.128$). There was reduced but measurable growth at pH 9 ($OD_{600} = 0.090 \pm 0.092$) and pH 5 ($OD_{600} = 0.060 \pm 0.058$). Growth was markedly limited at pH 3 ($OD_{600} = 0.020 \pm 0.021$) and pH 11 ($OD_{600} = 0.030 \pm 0.028$), with no growth observed at pH 1.

Salt tolerance testing demonstrated optimal growth at 1% NaCl ($OD_{600} = 0.130 \pm 0.128$), with moderate growth

observed in the absence of added salt (0% NaCl: $OD_{600} = 0.090 \pm 0.092$) and at 5% NaCl ($OD_{600} = 0.090 \pm 0.092$). Growth was significantly diminished at 3% NaCl ($OD_{600} = 0.011 \pm 0.012$) and 7% NaCl ($OD_{600} = 0.040 \pm 0.038$), and was entirely inhibited at 11% NaCl.

Bile salt tolerance constitutes a vital characteristic of probiotic bacteria. The isolate S9B43 demonstrated exceptional resilience to bile salts across a broad concentration spectrum. The growth rate exhibited a progressive increase from 0% to 2% bile salts, with the peak growth observed at 2% ($OD_{600} = 0.360 \pm 0.362$). Furthermore, growth at concentrations of 0.3% ($OD_{600} = 0.170 \pm 0.172$), 0.5% ($OD_{600} = 0.210 \pm 0.208$), and 1% ($OD_{600} = 0.280 \pm 0.278$) demonstrated a dose-dependent enhancement. Even at 3% bile salts, growth remained substantial ($OD_{600} = 0.240 \pm 0.238$), indicating a robust tolerance to elevated bile levels that significantly surpass physiological concentrations within the crustacean gastrointestinal tract.

Table 5: Environmental tolerance of isolate S9B43

Parameter	Condition / Agent	Result (OD_{600})
Temperature (°C)	0	0.000±0.000
	4	0.090±0.092
	15	0.100±0.098
	20	0.110±0.108
	30 (optimal)	0.140±0.138
	37	0.090±0.092
	45	0.040±0.038
	80	0.020±0.018
pH	1	0.000±0.000
	3	0.020±0.021
	5	0.060±0.058
	7 (optimal)	0.130±0.128
	9	0.090±0.092
	11	0.030±0.028
NaCl concentration (%)	0	0.090±0.092
	1 (optimal)	0.130±0.128
	3	0.011±0.012
	5	0.090±0.092
	7	0.040±0.038
	11	0.000±0.000
Bile salt concentration (%)	0	0.130±0.128
	0.3	0.170±0.172
	0.5	0.210±0.208
	1	0.280±0.278
	2 (maximum growth)	0.360±0.362
	3	0.240±0.238

Discussion

Taxonomic Identity and Biochemical Profile

The current investigation has effectively isolated a cultivable bacterium associated with the gut, identified as S9B43, from the intestinal tract of a healthy wild *Metapenaeus dobsoni*. Wild aquatic organisms host a diverse microbiota within their intestines, which play roles in nutrient metabolism, immune regulation, and colonisation resistance, thereby serving as valuable reservoirs of functionally significant bacteria [42-45].

The phenotypic and biochemical characterization indicated that isolate S9B43 exhibited metabolic features characteristic of members of the tribe Pseudomonadata, including positive reactions for indole production,

phenylalanine deaminase activity, nitrate reduction, hydrogen sulphide production, and oxidative-fermentative metabolism. Nevertheless, ABIS Online tentatively identified the isolate as *Pseudomonas pseudoalcaligenes* with only 45.24% probability, illustrating the limited discriminatory capacity of phenotypic identification methods for environmental isolates [46-48].

Conversely, 16S rRNA gene sequencing identified isolate S9B43 as *Morganella morganii* with 100% sequence identity, and phylogenetic analysis corroborated its taxonomic classification within the *M. morganii* clade. Prior reports have documented similar inconsistencies between biochemical and molecular identification methods, thereby

emphasising the critical role of molecular techniques in the precise identification of environmental bacteria [47-49].

Although *M. morganii* is broadly acknowledged as an opportunistic bacterium, it also naturally resides within the gastrointestinal tract of fish and crustaceans [50]. The isolation of S9B43 from a healthy wild shrimp indicates that it may constitute part of the native gut microbiota rather than being solely an opportunistic organism. However, additional genomic and safety assessments are necessary to elucidate its ecological role and functional importance [50, 51].

Environmental Stress Tolerance and Gastrointestinal Survival

Isolate S9B43 exhibited considerable tolerance to varying environmental conditions, thriving within a temperature range of 4-45°C with optimal growth observed at 30°C. This aligns with the tropical habitat of *Metapenaeus dobsoni* and the mesophilic nature of gut-associated bacteria [52, 53]. Although limited growth was observed at 80°C, this finding is atypical for non-thermophilic bacteria and merits further investigation prior to drawing any biological conclusions.

The isolate tolerates a broad pH spectrum [3-11], exhibiting optimal growth at pH 7. No growth was observed at pH 1. This tolerance indicates an adaptation to the variable physicochemical conditions present within the shrimp gastrointestinal tract and aligns with prior studies documenting extensive pH tolerance among host-associated aquatic bacteria [54-56].

Isolate S9B43 also demonstrated moderate halotolerance, exhibiting optimal growth at 1% NaCl and sustaining growth at salinity levels up to 7% NaCl, although growth was entirely inhibited at 11% NaCl. Such salinity tolerance is beneficial for bacteria residing in estuarine and coastal habitats, where salinity levels vary significantly [57, 58].

A notable characteristic of isolate S9B43 was its capacity to tolerate bile salts up to 3%, with peak growth observed at 2% bile salts. Given that bile tolerance is regarded as a critical prerequisite for persistence within the gastrointestinal tract, this trait may augment the isolate's ability to survive and compete within the shrimp gut environment. Nevertheless, the atypical increase in growth at higher bile concentrations necessitates further physiological investigation to elucidate the underlying mechanisms [59, 60].

Pathogen Inhibition Mechanisms

The antibacterial activity of *Morganella morganii* S9B43 varied with both the test pathogen and the bacterial fraction tested. The cell-free supernatant inhibited *Aeromonas hydrophila*, whereas the crude cell lysate inhibited *Vibrio parahaemolyticus*, *Edwardsiella tarda*, and *Streptococcus iniae*. These findings suggest that the isolate produces antimicrobial compounds with distinct cellular localisations, potentially contributing to competitive interactions within the shrimp gut microbiota [61, 62].

The inhibition of these pathogens is particularly significant, given that they are among the principal bacterial agents responsible for motile aeromonad septicaemia, vibriosis, edwardsiellosis, and streptococcosis in aquaculture, which result in substantial economic losses worldwide [35, 63]. Comparable antagonistic activity against pathogens affecting fish and shrimp has been documented for various host-associated bacteria, underscoring the role of intestinal

microbiota in preserving microbial homeostasis and inhibiting opportunistic pathogens [10, 61, 64].

Although the present study did not characterise the antimicrobial compounds responsible for pathogen inhibition, the observed activity demonstrates the functional potential of isolate S9B43. Further studies are required to identify the bioactive metabolites and elucidate their modes of action.

Safety Considerations

The safety of candidate host-associated bacteria is a critical prerequisite for their potential use in aquaculture. In the present study, isolate S9B43 exhibited α -haemolytic activity, characterised by partial haemolysis of erythrocytes. Although α -haemolysis is generally considered less virulent than β -haemolysis, it is not definitive evidence of safety, particularly for members of the genus *Morganella*, which includes opportunistic pathogens [65, 66]. Consequently, haemolytic activity should be interpreted alongside additional safety assessments rather than as an independent criterion.

The isolate also demonstrated resistance to ampicillin, erythromycin, and oxytetracycline. While some level of antimicrobial resistance is inherently present among Gram-negative enteric bacteria, the occurrence of resistance, particularly to oxytetracycline, necessitates meticulous evaluation due to its extensive application in aquaculture [67]. Current international guidelines advise that bacterial strains intended for probiotic or biotechnological use should undergo assessment for the presence of transferable antimicrobial resistance determinants and virulence-associated genes prior to deployment [36, 68]. Consequently, whole-genome sequencing, antimicrobial resistance gene profiling, and *in vivo* safety assessments are essential to ascertain whether the observed resistance is intrinsic or linked to mobile genetic elements.

Functional Significance and Future Perspectives

The present study demonstrates that *Morganella morganii* S9B43 exhibits several functional characteristics, including antagonistic activity against major aquaculture pathogens and tolerance to a broad range of environmental conditions, which may promote its persistence within the shrimp gastrointestinal tract [10, 21, 69]. Furthermore, the isolation of the bacterium from the intestine of a healthy wild *Metapenaeus dobsoni* suggests that it constitutes part of the indigenous gut microbiota and underscores wild aquatic organisms as valuable reservoirs of functionally important bacteria.

Despite these promising *in vitro* characteristics, the observed α -haemolytic activity and antibiotic resistance underscore that functional attributes alone are inadequate to determine probiotic suitability. A comprehensive genomic analysis, encompassing screening for virulence and antimicrobial resistance genes, along with adhesion studies and *in vivo* feeding and challenge experiments, is imperative to validate the safety, colonisation capacity, and functional efficacy of isolate S9B43 prior to any practical application [6, 36, 68, 69].

The findings of this study enhance the existing knowledge regarding the cultivable gut microbiota associated with wild *M. dobsoni* and establish a basis for subsequent research on the ecological functions and diversity of host-associated bacteria in shrimp.

Conclusion

The present study successfully isolated and characterized a cultivable gut-associated bacterium, *Morganella morganii* S9B43, from the intestine of a healthy wild *Metapenaeus dobsoni*. While conventional phenotypic and biochemical analyses provided preliminary taxonomic information, 16S rRNA gene sequencing confirmed the identity of the isolate, emphasising the significance of molecular methodologies for precise identification of environmental bacteria.

Isolate S9B43 displayed multiple functional traits, such as antagonistic activity against key aquaculture pathogens and resilience to various environmental conditions, signifying its adaptation to the gastrointestinal environment of shrimp. Nevertheless, the detected α -haemolytic activity and resistance to antibiotics underscore the necessity for additional genomic, safety, and *in vivo* studies prior to any practical implementation.

Overall, this study enhances the existing knowledge regarding the cultivable gut microbiota linked with wild *M. dobsoni* and recognises *M. morganii* S9B43 as a host-associated bacterium with significant functional implications, thereby offering a valuable foundation for forthcoming investigations into the ecological roles and functional diversity of native gut bacteria in sustainable aquaculture.

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