

Article

Development of New Drug Against Multidrug-Resistant *Candidozyma (Candida) auris* by Mining the Genome of Marine Bacteria *Vibrio* sp. IRMCESH58L

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Abstract

Background/Objectives: *Candidozyma auris* is the most frequent multidrug-resistant fungal infection in the Arabian Peninsula, with high mortality rates; therefore, new medications are in high demand. Microbes in marine habitats have genetically evolved to survive under a variety of adverse conditions, including severe temperatures, salinity, pH, and other stress factors, by generating various bioactive metabolites. These bioactive secondary metabolites have strong potential for use as antifungal agents. Due to the shortage of antifungal medications and the emergence of treatment resistance in *C. auris*, identifying new therapeutics from synthetic bacterial components or natural materials has become a necessity. Natural molecules have numerous advantages over synthetic substances, including structural variation and low toxicity. Few next-generation sequence-based investigations have been carried out on anti-*Candidozyma auris* bacterial species to identify potential therapeutic candidates. Therefore, the aim of this study is to identify biosynthetic gene clusters from marine bacteria using next-generation sequencing to discover novel drug compounds against multidrug-resistant *C. auris*. **Methods:** More than 68 isolates were collected from various marine environments using standard techniques. All isolates were tested against the multidrug-resistant *C. auris*. Scanning electron microscopy was utilized to investigate the cell membrane rupture caused by defused metabolites of the IRMCESH58L bacterium in *C. auris*. The *Vibrio* sp. IRMCESH58L genome was sequenced using long-read nanopore sequencing technology. **Results:** The bacterial strain IRMCESH58L, isolated from a fish liver sample, showed the highest and most constant activity against *C. auris*. An in vitro toxicity test found that IRMCESH58L had no cell cytotoxicity against HFF-1 cells. The assembled plasmid-free genome is 6,556,025 bp (48.93% G+C),



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with an N50 of 909243. Comparative analysis confirmed its relation to *Vibrio alginolyticus*. **Conclusions:** Whole-genome analysis of the native bacterial strain IRMCESH58L revealed various biosynthetic gene clusters, including those involved in surfactin's biosynthesis of putative natural anti-*C. auris* chemicals, but no pathogenic protein-coding genes, emphasizing the importance of marine bacteria in the fight against *C. auris*. Following this in vivo study, therapeutic targets will later be selected for further pre-clinical studies.

Keywords: *Candidozyma auris*; multidrug-resistant fungi; marine bacteria; secondary metabolites; next-generation sequencing; antifungal agents; drug discovery

1. Introduction

Candidozyma auris (*Candida auris*) is a multidrug-resistant fungus. The name is taken from the Latin word for the ear (auris). *C. auris* was initially discovered in 2009 in a Japanese patient's external ear canal [1]. Following its discovery, this fungal pathogen was divided into four distinct clades, each of which has been found to have spread over the world in diverse medical settings [2]. Patients with various underlying medical conditions are primarily infected in the bloodstream by the nosocomial spread of *C. auris*. Due to the drug-resistant nature of *C. auris*, the three antifungal drug classes developed high-risk invasive fungal candidemia [3]. The mortality rate ranges from 30 to 60% as a result of both patient comorbidities and the drug resistance of *C. auris* [4]. The majority of instances that have been reported over the past five years have been isolated from blood and other deep-seated infection sites, such as catheter tips and deep infections. Numerous clinical conditions have been linked to *C. auris*, including myocarditis, meningitis, bone infections, wound infections, urinary tract infections, otitis, surgical wound infections, and skin abscesses related to catheter insertion [4]. *C. auris* is frequently misidentified as *Candida haemulonii* by laboratory identification systems (i.e., the VITEK 2 system) [5]. For accurate microbial antibiotic susceptibility testing, bacterial proteins from whole-cell extracts have recently been profiled using Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) to create a bacterial fingerprint that can distinguish between microorganisms of various genera and species. It is more precise than alternative tests for detecting *C. auris* and enables later epidemiological strain characterization. Although MALDI-TOF MS systems have obtained FDA approval and can be used to precisely and swiftly detect *C. auris*, they still present challenges due to the high cost of the equipment [6]. Refining MALDI-TOF MS databases is essential for achieving higher diagnostic precision [7].

The emergence of the MDR fungal pathogen *C. auris* has necessitated the discovery of new drugs and the innovative development of target therapies. In vitro research has helped to identify a large number of inhibitory substances, for example, SCY-078, which is a triterpene glucan synthase inhibitor that has shown action against some of the most common *Candida* species, including echinocandin-resistant isolates, both in vitro and in vivo, as well as all *C. auris* clades [8]. Another study revealed how the new arylamidine (T-2307) functions, reporting that a new arylamidine, currently undergoing clinical trials, could be effective against fungal mitochondria. T-2307 has shown powerful in vitro and in vivo activity against most fungi. Similar research involved the screening of a new drug, SCY-247, that has major antifungal properties against yeast and mold strains. Initial findings demonstrated that SCY-247 was effective against several fungal isolates. The antifungal effects of SCY-247 were further investigated against strains of *Candida albicans*, *Candida glabrata*, and *Candidozyma auris* [9]. Alternatives to antifungal drugs that can inhibit the growth of *C. au-*

ris have been proposed by drug repurposing approaches and nanomaterial-based studies, but new, promising drugs are still required for effective treatment. For example, a possible topical treatment option for superficial candidiasis is metal nanoparticles. Antifungal effectiveness and cytotoxicity traits must be enhanced and modified for therapeutic uses [10]. Research to identify antifungal microorganisms, particularly for *Candidozyma auris*, is ongoing. The aim of our current research is to find natural substances that can be used for drug-resistant fungi, using marine bacteria as an essential anti-*Candida* agent.

In Africa, the Middle East, South America, and other regions of the world, cases of *C. auris* have slowly started to appear. At this point, *C. auris* cases are found on every continent. The Centers for Disease Control and Prevention (CDC) sent an issuance of clinical notice to healthcare facilities in the USA as a result of *C. auris* infection cases beginning to appear in the country for the first time in New York and spreading to many other states. The analysis of the *C. auris* isolates via whole-genome sequencing (WGS) revealed four distinct clades that originated in various geographical areas: East Asia, Africa, South Asia, and South America. A fifth clade, originating from Iran, was recently discovered. Single-nucleotide polymorphisms (SNPs) with thousands of variations revealed the clade varieties [11]. Nursing homes, long-term facilities, and intensive care units (ICU) are the main targets of the nosocomial spread of the multidrug-resistant *C. auris*. Most of the hospitalized patients with this fungal pathogen are typically immunocompromised, i.e., those in the intensive care unit, those with concomitant conditions, and those who have had a long hospital stay. The spread of *C. auris* infection may be attributed to its propensity to survive on a variety of dry and damp surfaces, including sinks, catheters, thermometers, beds, and linen, or even from surface to surface or patient to patient, according to a number of studies conducted in various healthcare institutions [12,13]. Patients with comorbidities, those who have spent a long time in the hospital, and those who have received antimicrobial therapeutics are also at risk. Unlike the more prevalent pathogenic fungi, *C. glabrata* and *C. albicans*, which are a part of the normal flora in the gastrointestinal track, *C. auris* is thought of as a high-risk pathogen because it easily spreads in the hospital environment and survives on most hospital surfaces [13]. Studies performed by different groups of researchers showed that chlorine-based disinfectants are the most effective at removing *C. auris* from hospital surfaces because they are not employed as often as ammonium compounds in hospitals [12].

The fact that *C. auris* exhibits variable drug resistance to three classes of antifungal drugs—i.e., polyenes, azoles, and echinocandins—that are currently used to treat candidemia is particularly concerning. Various geographical clades of fungal pathogens have been found to exhibit wavering treatment-level resistance to various antifungal medication classes [14]. Drug resistance is partly related to the initial antibiotic regimen used for antifungal medications in treating fungal infections prior to correctly identifying the causative fungal organism, even if the mechanisms of resistance are still being researched [15]. Fluconazole is the primary option for treating *Candida* infections and is widely available in developing countries. Studies on drug susceptibility have shown that most of the isolates of *C. auris* exhibit high azole resistance [16]. However, because not all geographically distinct *C. auris* isolates share the same fluconazole resistance, and because the isolates' mutations vary in the *ERG11* gene, genome sequencing analysis of these isolates has revealed an acquired mechanism that is associated with fluconazole resistance. Although the isolates of *C. auris* exhibit resistance to other azoles, their minimum inhibitory concentrations are lower than those of fluconazole. Various clades of polyene drugs, like Amphotericin B, display different drug susceptibility traits. Another study collected six samples from blood, urine, and an ear swab and tested them for antifungal susceptibility. All the fungal isolates were fluconazole-resistant. Sanger sequencing of the isolates' *FKS1* and *ERG11*

genes showed that *ERG11* had two mutations (F132Y and K143R) linked to drug resistance, whereas *FKS1* had no mutations. The study highlighted the danger that an emerging pathogen poses to public health, and hospital settings have put strict infection control and contact screening measures in place to guard against *C. auris* infection. Lastly, it is imperative to keep track of antifungal resistance in various regions and apply effective treatment protocols [17].

A systematic review looked at 11 studies to determine how common *C. auris* was in clinical samples from Kuwait, Saudi Arabia, and the United Arab Emirates. It identified 580 strains. In a systematic review study from the Arab region, six human isolates of *C. auris* were reported in the axilla, groin, anterior nares, oropharynx, respiratory, vascular line exit sites, and urinary tracts. As environmental samples for the isolates, all the *C. auris*-infected units, colonized patient rooms, medical equipment, flooring, furniture, linen, walls, doorknobs, bed rails, toilet faucets, bedside drawers, and flush handles were considered [18]. The most common MDR fungal infection in the Arabian Peninsula, *C. auris* (580 strains), is linked to 54 fatalities [18]. Next-generation sequence-based studies on the anti-*Candida auris* bacterial organisms of marine origin are not available in Saudi Arabia. Hence, the objective of this study is to discover and characterize anti-*C. auris* bacteria from the marine environment using *16S rRNA* sequencing, followed by whole-genome sequencing and computational analysis to identify specific biosynthetic gene clusters and potential drug candidates for the treatment of *C. auris* infection. To address the urgent need for novel therapies against multidrug-resistant *C. auris*, this study systematically screened 64 diverse marine samples from Eastern Saudi Arabia to isolate and genomically characterize potent antagonistic bacteria, specifically identifying the secondary metabolite potential of *Vibrio* sp. IRMCESH58L.

2. Material and Methods

2.1. Isolation of Bacteria

To find bacteria with anti-*C. auris* activity, an examination of several marine samples from various sources was carried out. All samples were processed in the enrichment medium—trypticase soy broth (TSB, MoleQule-On, Auckland, New Zealand). After the observed turbidity in the broth medium (TSB), streaking techniques were performed on trypticase soy agar (TSA, MoleQule-On, Auckland, New Zealand) and incubated at 37 °C for 24 h. The colony characteristics were evaluated, and isolated pure colony cultures were placed in a cryotube with glycerol and TSB and stored at −80 °C.

2.2. Anti-*Candidozyma auris* Activity Screening

Before evaluating the effectiveness of the bacteria against *C. auris*, 0.5 McFarland's standard for *C. auris* was achieved for both bacteria and fungi. The Kirby–Bauer disk diffusion method was undertaken to detect *C. auris* activity. The bacterial isolate IRMCESH58L was prepared for anti-*C. auris* activity screening using a pure colony after 24 h of incubation in TSB media. The IRMCESH58L was washed twice with PBS (phosphate-buffered saline) and then spun via a centrifuge to suspend the cell pellet to obtain a high bacterial concentration. Then, 100 µL of the concentrated bacterial suspension was placed on a disk. The disk was placed on Sabouraud dextrose agar (SDA) (MoleQule-On, Auckland, New Zealand) that was wiped by *C. auris* and incubated at 37 °C for 24 h. The antifungal susceptibility of bacteria to *C. auris* was determined by measuring the zone of inhibition.

2.3. IRMCESH58L and *Candidozyma auris* Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) (Vega 3, TESCAN, Brno, Czech Republic) was used to visualize *C. auris* and IRMCESH58L. One colony of *C. auris* and one colony of

IRMCEH58L were taken from a 24 h old culture on an agar plate. A total of 500 µL of 3% glutaraldehyde was added, and the tubes were incubated at 4 °C for 16 h before being washed three times with distilled water for 10 min each time. Samples were treated with acetone (in series concentrations of 30%, 50%, 70%, and 100%) for 10 min after being fixed at 1% osmium tetroxide (OsO₄) for 16 h at 4 °C. Finally, the samples were kept in absolute acetone to dry. Acetone and carbon dioxide were added to dry the sample. The sample was mounted onto a metallic stub using double-sided carbon tape and sputter coated with gold (Q150R ES machine). A scanning electron microscope (Vega 3, TESCAN, Brno, Czech Republic) running at 20 kV was used to observe *C. auris* and IRMCEH58L cells. The digital imaging program Image 1.53t (<https://imagej.nih.gov/ij/>) (accessed on 19 March 2023) was used to measure the cells. The zone of inhibition was scanned after 24 h of IRMCEH58L treatment against *C. auris* using the Kirby–Bauer disk diffusion method. *C. auris* from the edge of the zone of inhibition (T1), *C. auris* from the zone of inhibition (T2), *C. auris* from the fungal growth region (T3), and IRMCEH58L growth from the disk (T4) were all examined using SEM. The zone of inhibition cells T1, T2, T3, and T4 were scraped and collected, and cultures were prepared for scanning by SEM to assess the interaction between *C. auris* and marine bacteria IRMCEH58L. The 1.53t software was used to examine the SEM images.

2.4. MTT Assay

Cell viability was assessed by the MTT assay (in triplicate). In our study, Human Foreskin Fibroblast (HFF-1) (ATCC[®] SCR-C-1041[™], Manassas, VA, USA) was used post-exposure to assess the cytotoxicity of IRMCEH58L 24 h cultured broth. The HFF-1 cells were seeded in a 96-well plate with a seeding density of 13,000 cells/mL for 24 h before applying the control to bacteria-free Tryptic Soy Broth (TSB) (control) (MOLEQULE-ON, Auckland, New Zealand), and IRMCEH58L broths were added to the cells. Both testing broths were diluted in Dulbecco's Modified Eagle's Medium (DMEM, Gibco[™] ThermoFisher Scientific, Waltham, MA, USA) to prepare the diluents at 50%, 40%, 20%, 10%, 5%, and 1%. These diluents were then incubated with HFF-1 cells for 24 and 48 h. To prepare the cells for the MTT assay, they were washed once with 1 × PBS (phosphate-buffered saline); then, fresh DMEM was added to each well, in addition to 10 µL of the 5 mg/mL MTT reagent (Sigma-Aldrich (St. Louis, MO, USA), cat. no. M2128), to reach 0.5 mg/mL as a final concentration in each well of the 96-well plate. The plate was incubated for 4 h in a humidified 5% CO₂ atmosphere at 37 °C, as recommended in the MTT manufacturer's manual. After 4 h, the media was discarded, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan product. The absorbance was measured at 570 nm using a Synergy NEO2 plate reader (Biotek Instruments, Winooski, VT, USA). Statistical analysis for the MTT cytotoxicity assay was performed using two-way ANOVA with the Bonferroni multiple-comparison test in GraphPad Prism software, version 10.6.1 (799) (GraphPad Software, La Jolla, CA, USA). A *p*-value of <0.05 was considered significant (* if *p* ≤ 0.05; ** if *p* ≤ 0.01; *** if *p* ≤ 0.001; **** if *p* ≤ 0.0001). The treated samples' absorbance readings were normalized to the TSB-treated TSB, where they present 100% cell viability according to the following formula:

$$\text{Cell viability (\%)} = \frac{\text{Sample}_{\text{Abs}}}{\text{Control}_{\text{Abs}}} \times 100$$

2.5. Cell Imaging

Cell morphology was evaluated using an inverted light microscope (T100F Eclipse, Nikon, Japan) combined with the MTT cytotoxicity assay to observe morphological changes associated with the cytotoxic effects of IRMCEH58L on HFF-1 cells. The plates were carefully examined under a standard light microscope at a 100× magnification to assess alterations in cell structure and adhesion.

2.6. DNA Extraction and 16S rRNA Gene PCR and Sequencing of IRMCESH58L

Total genomic DNA from IRMCESH58L was extracted at the Institute for Research and Medical Consultations (IRMC) using a Qiagen Yeast/Bacteria Kit (Gentra Puregene Yeast/Bact. Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The 16S rRNA gene (~1400 bp) of the IRMCESH58L strain was amplified using 16S rRNA F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 16S rRNA R (5'-TAC GGC TAC CTT GTT ACG ACT T-3') (MoleQule-On, Auckland, New Zealand) at an annealing temperature of 61.8 °C using absolute master mix (MoleQule-On, Auckland, New Zealand) in a professional thermocycler (Biometra, Göttingen, Germany) for 35 cycles.

Following product visualization on a 2% agarose gel, the PCR-amplified DNA was purified using a QIAquick PCR Purification Kit (Qiagen, Germany). Forward and reverse primers were used for amplification using the Big Dye® Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and the purified amplified DNA was sequenced via 3500 genetic analyzers (Applied Biosystems, Foster City, CA, USA). Whole-genome sequencing of marine bacteria was performed to identify genes with bioactive metabolites.

2.7. Library Preparation and Nanopore Whole-Genome Sequencing

Input gDNA samples were ensured to be chemically pure using a nanodrop spectrometer (NanoDrop™ 8000 Spectrophotometer, Thermo Scientific U.S.A., Waltham, MA, USA) with 260/280 nm ratios between 1.8 and 2, 260/230 nm ratios between 2.0 and 2.2, and a concentration of at least 100–500 ng in a 50 µL volume.

2.7.1. DNA Repair and End Preparation

The NEBNext FFPE DNA Repair Mix and NEBNext Ultra II End Repair/dA-Tailing Module reagents (New England Biolabs) were thawed, vortexed thoroughly, and briefly centrifuged using a Gilson™ GMCLab™ (Middleton, WI, USA) mini-centrifuge before being maintained on ice. Genomic DNA (gDNA) volume was adjusted to 48 µL using nuclease-free water, followed by pulse-vortexing and brief centrifugation. The end repair reaction was prepared in a 0.2 mL PCR tube. Components were mixed by gentle flicking and pulse-centrifuged. The mixture was incubated in an Eppendorf™ Mastercycler™ Nexus Thermal Cycler at 20 °C for 5 min, followed by 65 °C for 5 min. Post-incubation, the DNA sample was transferred to a 1.5 mL DNA LoBind® tube (Eppendorf, Hamburg, Germany). Purification was performed by adding 60 µL of resuspended AMPure XP beads (Beckman Coulter, Brea, CA, USA) and incubating the mixture on a MaxiMix™ II rotator (Thermo Scientific, USA) for 5 min. The beads were pelleted using a MagAttract magnetic rack (Qiagen, Germany). The supernatant was discarded, and the pellet was washed twice with 200 µL of freshly prepared 70% ethanol. Residual ethanol was removed after a brief 30 s drying period. The DNA was eluted by resuspending the pellet in 25 µL of nuclease-free water, followed by a 2 min incubation period at room temperature. The beads were pelleted for 1 min until the eluate was clear. The final 25 µL eluate was recovered, and 1 µL was quantified using a Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Samples yielding > 700 ng of end-prepped DNA were prioritized for subsequent native barcoding and ligation.

2.7.2. Native Barcoding and Ligation

The native barcodes were thawed at room temperature. The individual barcodes were mixed by pipetting. They were spun down and placed on ice until they were ready to use, and 500 ng of each end-prepped sample was diluted to 22.5 µL using nuclease-free water. The barcoding mixture was prepared. The solution was mixed well by pipetting or

by gently flicking the tube and then spun down. The reaction mix was incubated for 10 min at room temperature. A total of 50 μL of resuspended AMPure XP beads was added, and the mixture was mixed by pipetting. The reaction mix was incubated on a rotator mixer for 5 min at room temperature. After incubation, the sample was spun down, and the pellet was collected on a magnet stand. The tube was kept on the magnet stand, the supernatant was pipetted off, and the pellet was washed with 200 μL of freshly prepared 70% ethanol without disturbing the pellet. The ethanol was pipetted off and discarded. The previous step was repeated. The tube was centrifuged briefly and then placed back onto the magnet stand. Any residual ethanol was pipetted off, and the pellet was allowed to dry for 30 s. The tube was removed from the magnet, and the pellet was resuspended in 26 μL of nuclease-free water. The mixture was incubated for 2 min at room temperature. The tube was placed back on the magnet stand until the eluate was clear and colorless. A total of 26 μL of eluate was removed and retained in a clean 1.5 mL tube. The pelleted beads were discarded, and 1 μL of the sample was quantified using a Qubit fluorometer. All barcoded samples were pooled sufficiently into a 1.5 mL DNA LoBind tube to produce a combined concentration of 700 ng total. A total of 1 μL of the pooled barcoded sample was quantified using a Qubit fluorometer, and 700 ng of the pooled barcoded sample was diluted to 65 μL in nuclease-free water. If 700 ng of the pooled sample exceeded 65 μL , an AMPure clean-up was performed with $2.5\times$ AMPure XP beads in the pooled sample volume, eluting 65 μL with nuclease-free water. The sample was stored at 4 $^{\circ}\text{C}$ overnight if there was not enough time to continue to the next step.

2.7.3. Adapter Ligation and Clean-Up

Elution Buffer (EB) and NEBNext Quick Ligation Reaction Buffer ($5\times$) were thawed and mixed with Adapter Mix II (AMII) and Quick T4 DNA Ligase to ligate the pooled and barcoded DNA. The clean-up procedure requires long-fragment buffer (LFB) or short-fragment buffer (SFB), depending on whether DNA fragments larger than 3 kb are to be enhanced or maintained. The reaction mix was incubated at room temperature for 10 min. A total of 50 μL of resuspended AMPure XP beads was added and incubated on a rotator mixer for 5 min at room temperature. The beads were pelleted on a magnet stand and then pipetted off the supernatant. The pelleted beads were washed with 250 μL of either long- or short-fragment buffer. The tube was spun down and placed back on the magnet stand. The supernatant was pipetted off, and this step was repeated. The tube was spun down and placed back on the magnet stand, and the residual supernatant was discarded. The sample was allowed to dry for 30 s. The tube was then removed from the magnet stand. The pellet was suspended in 15 μL of elution buffer (EB). The tube was spun down and incubated at room temperature for 10 min. Incubating at 37 $^{\circ}\text{C}$ improves long-term fragment recovery. The beads were pelleted on the magnet stand for 1 min or until the eluate was colorless. A total of 15 μL of the eluate was removed and placed in a clean 1.5 mL tube. A concentration of 1 μL of eluate was measured using a Qubit fluorometer. The recovery goal was 430 ng. The prepared library was then ready for flow cell loading.

2.7.4. Priming and Loading the SpotON Flow Cell

The SpotON Flow Cell (Oxford Nanopore Technologies-ONT, Oxford, UK) was acclimated at room temperature. The sequencing buffer (SQB), loading beads (LB), flush tether (FLT), and flush buffer (FB) were thawed and mixed via the vortexing method. The reagents were placed on ice. Then, we opened the MinION Mk1B lid and slid the flow cell beneath the clip. We opened the priming port of the flow cell and drew back a tiny volume to remove air bubbles. A total of 800 μL of priming mix was loaded into the flow cell via the priming port. We waited at least 5 min for the priming mix to be totally absorbed. The

library loading mix was prepared by mixing 37.5 µL of sequencing buffer (SQB), 25.5 of loading beads (LB), and 12 µL of the DNA library. We opened the SpotON sample port cover for accessibility. A total of 200 µL of priming mix was loaded via the priming port (not the SpotON sample port) to remove air bubbles. The library was gently mixed by pipetting up and down before loading. A total of 75 µL of the sample was loaded through the SpotON sample port drop by drop. The SpotON sample port and priming port were closed. The MinION lid (Oxford Nanopore Technologies-ONT, Oxford, UK) was replaced.

2.7.5. Data Acquisition and Base Calling

After setting all parameters according to the MinKNOW (Installed version: 23.04.6 Oxford Nanopore Technologies-ONT, Oxford, UK) setup and recommendations for sequencing using the MinION device, we used the MinKNOW software for control, data collection, and real-time base calling during the run. We analyzed the data using the EPI2ME (EPI2ME Agent 3.5.7, Oxford Nanopore Technologies-ONT, Oxford, UK) platform. Then, Oxford Nanopore tools were used to calculate the coverage and quality of the long reads generated. Then, we reconstructed the DNA sequence from the individual nucleotide signals and converted the sequences to fastQ files for analysis.

2.8. Genomic Data Analysis and Biosynthesis-Related Gene Cluster (BGC) Detection

The long-read sequence of IRMCESH58L was assembled and annotated using Nanoforms and NanoGalaxy, as described in previous studies [18,19]. The genome sequences of IRMCESH58L were uploaded to the Type Strain Genome Server (TYGS) and the Pathosystem Resource Integration Center (PATRIC) to perform genome-based taxonomic classification. A phylogenetic tree of IRMCESH58L was constructed using the whole genome [20,21]. The assembled fastQ files were subjected to genome analysis and comparison [21]. To visualize all gene names and features in open reading frames, genome mapping was used. The concatenated genomic DNA sequences were aligned using the Mauve algorithm to compare the genomic rearrangements among the anticipated species. PATRIC was also used to create a whole-genome phylogenetic tree with >50 single-copy genes aligned with >75 genomes from the genus *Vibrio* [22]. Plasmid multilocus sequence typing was used to detect plasmid types of IRMCESH58L [23]. Pathogenic protein families in IRMCESH58L were analyzed using PathogenFinder [24].

To identify the antifungal chemicals produced by the strain IRMCESH58L, two software programs, antiSMASHs 6.1.1 and MIBiG Version 3.1, were used [25]. Hidden Markov models (HMMs) were used to detect a diverse range of BGCs in the IRMCESH58L genome. The natural product domain seeker (NAPDOS 2) bioinformatic server was used with the genome data of the IRMCESH58L strain to identify BGC domains and their predicted structures [26]. Metabolic pathways between isolate and reference organisms were compared and limited to 2 closely related strains [25]. The metabolite profile of *B. Vibrio* sp. IRMCESH58L was analyzed via liquid chromatography–mass spectrometry (LC/MS) to confirm the production of secondary metabolites predicted by in silico genome mining.

3. Results

In the present study, we collected various samples from the marine environment, including sea water, sea water with algae, and fish from the marine region. We also collected various types of samples from the seashore between Qatif and Khobar (Table S1). All samples were collected in sterile containers and brought to the lab immediately for processing. Based on their nature, samples were subjected to bacterial isolation; in the case of the liquid samples, they were directly added to the enrichment medium to enrich the bacteria. Different types of bacterial isolates were isolated from the samples collected from

the marine environment using various media, amounting to a total of 64 bacterial strains (Table S1). These strains were coded as IRMCESH1 to IRMCESH64, serially, for further screening and analysis. Mixed colonies isolated from the marine samples were subjected to purification to isolate pure colonies from the mixed colonies, and they were stored at -20°C for further processing via primary anti-*C. auris* screening. A representative sample of the fish and fish liver used for the isolation of bacteria and an SEM image of the isolated bacteria (IRMCESH58L) are presented in Figure 1.

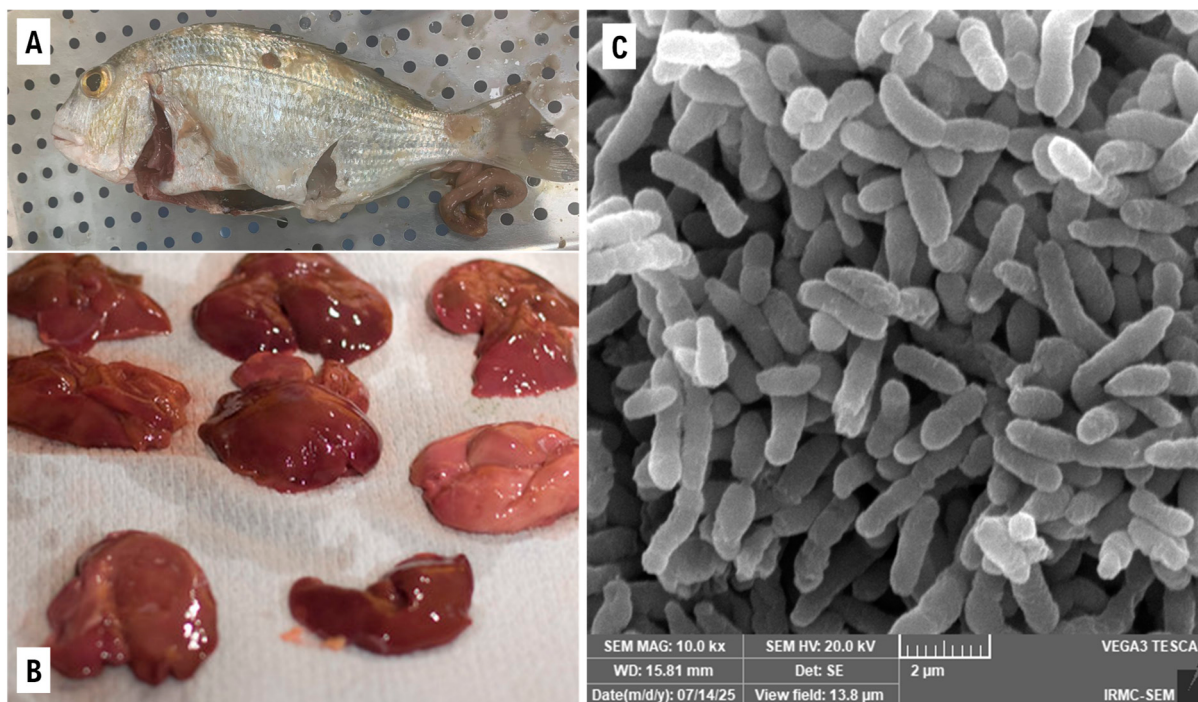


Figure 1. Source and structure of IRMCESH58L. (A,B) Fish and livers of fish used for the isolation of bacteria. (C) Scanning Electron Microscopy of IRMCESH58L.

The isolates (IRMCESH58L) obtained from fish liver included three distinct bacterial colonies, colored cream to white, while some were translucent and contained moisture. In addition, they included round flat colonies with smooth margins, as well as a few large bacterial isolates of approximately 4 mm, which were translucent, and isolated as a pure colony. All other isolated strains were similarly processed for primary screening.

3.1. Primary Screening of Bacteria with Anti-*C. auris* Activity

C. auris strains were collected from King Fahd Hospital of the University (KFHU), Khobar, Kingdom of Saudi Arabia. *C. auris* strains were cultured routinely in Sabouraud dextrose broth (SDB) or Sabouraud dextrose agar (SDA). Pure *C. auris* (length: 1793.589 nm; width: 773.0145 nm) strains were measured using electron microscope analysis (Figure 2), placed in a cryotube with glycerol, and stored at -80°C . All marine bacterial isolates were subjected to bacterial identification with anti-*Candida auris* activity using pure *C. auris* strains (Figure 2). Based on the susceptibility test, most of the isolates were observed to have no activity against *Candidozyma auris* (Figure 3), except the following: IRMCESH39, IRMCESH42, and IRMCESH58L. The IRMCESH58L showed the highest antifungal activity against *C. auris* and exhibited the same during repeated screening. It was also found that IRMCESH39 was moderately active. The inhibition zone around the disk was measured to record the anti-*C. auris* activity of the bacterial isolates. According to the results of the disk diffusion experiment, the bacterial isolate IRMCESH39-4D exhibited a weak inhibitory effect against *C. auris*.

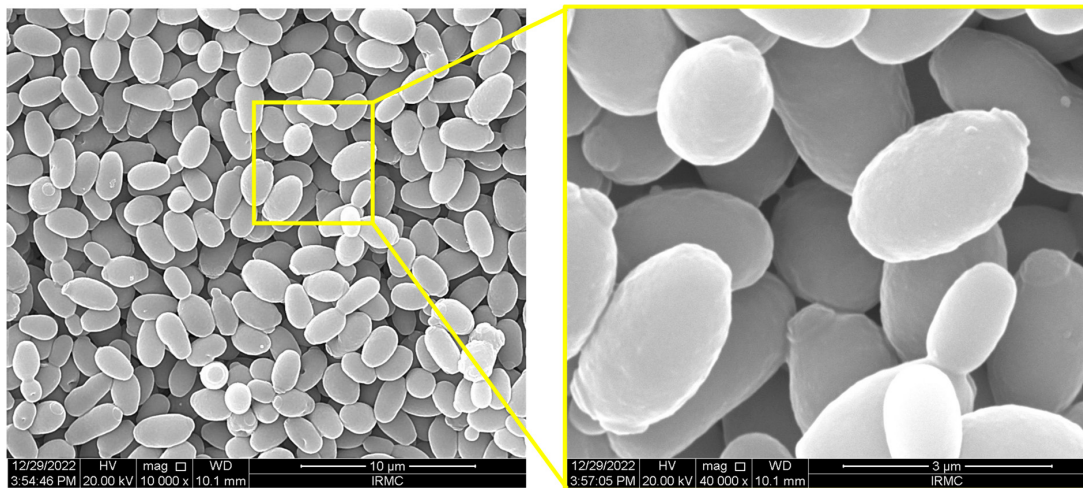


Figure 2. Pure *C. auris* strains used for the study. **(Left)** *C. auris* strains at the magnification of 10,000 $\times$. **(Right)** *C. auris* strains at the magnification of 40,000 $\times$.

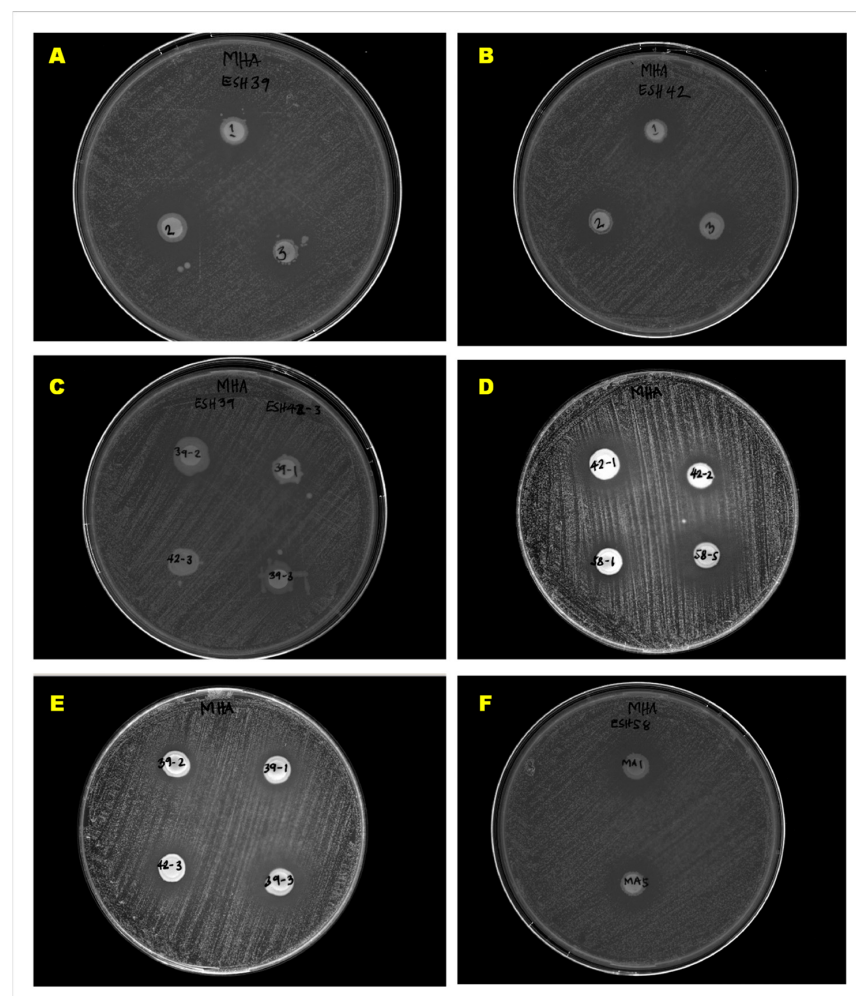


Figure 3. Primary screening of anti-*Candida* activity: **(A)** (IRMCESH 39-1) 13 mm; (IRMCESH39-2) 18 mm; (IRMCESH39-3) 16 mm. **(B)** (IRMCESH42-1) 15 mm; (IRMCESH 42-2) 18 mm; (IRMCESH42-3) 19 mm. **(C)** (IRMCESH39-2) no zone; (IRMCESH39-1) no zone (IRMCESH39-3) no zone; (IRMCESH42-3) no zone. **(D)** (IRMCESH39-2) 12 mm; (IRMCESH39) 10 mm; (IRMCESH42-3) 13 mm; (IRMCESH39-2) 15 mm. **(E)** (IRMCESH58L) 17 mm; (IRMCESH58L) 17 mm; (IRMCESH58L) 19 mm. **(F)** Antifungal activity follow-up of the zone inhibition IrmCesh 58L (MA1) 16 mm; IrmCesh 58L (MA5) 15 mm.

3.2. IRMCESH58L with Anti-*C. auris* Activity

The ability of the bacterial strain IRMCESH58L to inhibit the growth of *C. auris* was re-evaluated on SDA media after 48 h. The bacterial strain IRMCESH58L was isolated from the liver of a fish collected from the Dammam region of Saudi Arabia. Analysis revealed that the IRMCESH58L strain inhibited *C. auris* growth, with a zone of inhibition of 20 mm (Figure 4). On trypticase soy agar (TSA) medium, the IRMCESH58L bacterial strain had the characteristics of a sizable, opaque, and moist colony. *C. auris* cells are oval-shaped when examined via SEM.

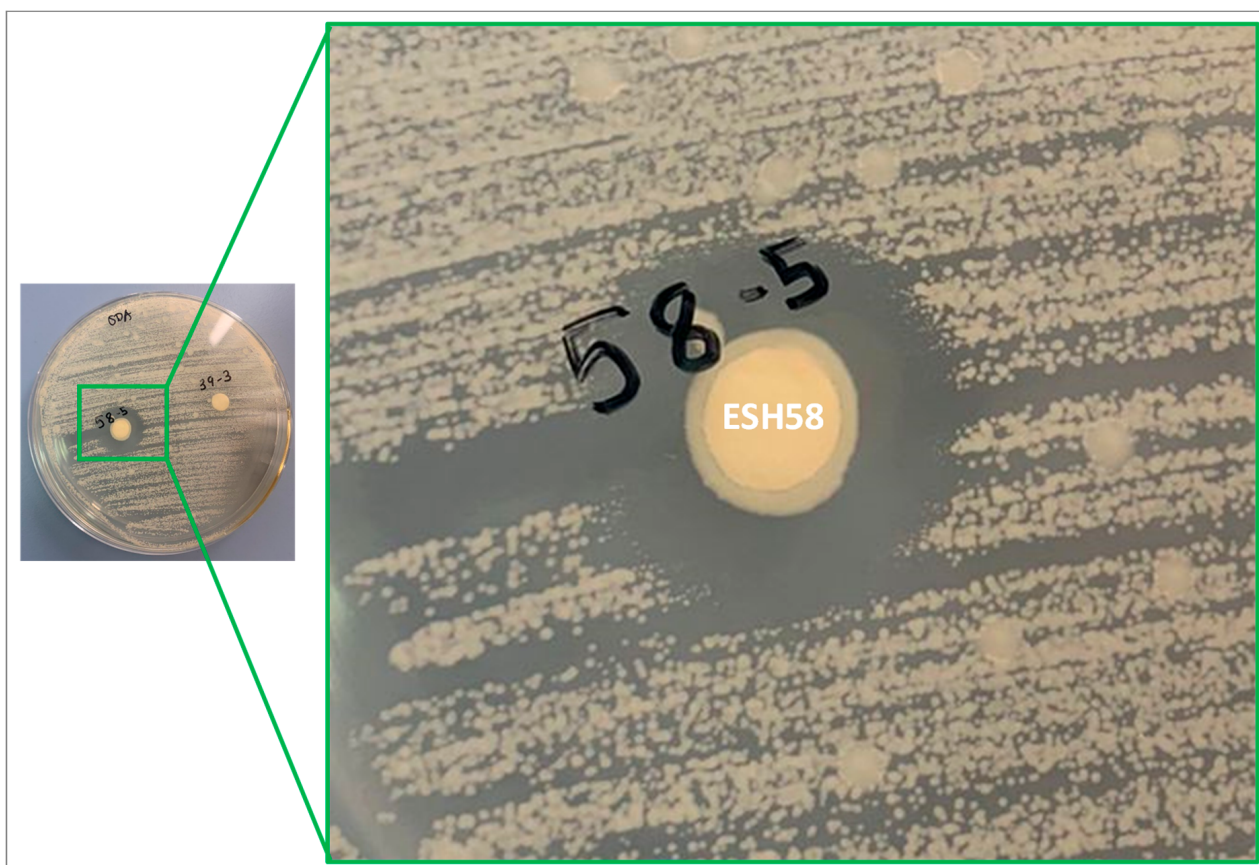


Figure 4. The best bacterial strain (IRMCESH58L) selected from the isolates of marine origin for antifungal activity, indicating the zone of inhibition.

3.3. Effect of IRMCESH58L on *C. auris* Using SEM

The Kirby–Bauer disk diffusion assay, which indicated successive zones of inhibition against *C. auris* during repeated screening, was used to determine the morphological effects of IRMCESH58L on *C. auris* using electron microscopic structural analysis. *C. auris* cells from the edge of the zone of inhibition [T1], the defused IRMCESH58L bacterium metabolites from the yeast cells evaluated from the zone of inhibition [T2], and *C. auris* cells in the typical fungal growth region [T3] all clearly demonstrate the impact of cell membrane disruption on the zone of inhibition (Figure 5). The *C. auris* cells from the normal growth zone exhibit a well-defined normal shape. Cell membrane disruption was identified via SEM examination of the *C. auris* zone of inhibition [T2]. While the normal structure of IRMCESH58L revealed anti-*C. auris* activity, the defused metabolites of the IRMCESH58L pathogeny on *C. auris* were revealed by the presence of disturbed cell membranes and lysed yeast cells, whereas the *C. auris* phenotype was smooth in the healthy cells and crushed in the IRMCESH58L-induced *C. auris* cells. Scanning electron micrographs revealed that the *C. auris* was smooth in the uninfected cells. *C. auris* cells after the treatment

[T2] clearly indicate the cell membrane disruption effect of the defused metabolites of IRMCESH58L bacteria on the yeast cells examined from the edge of the inhibition zone [T1]. Pathogenicity of IRMCESH58L on *C. auris* was observed by the presence of disturbed cell membranes and lysed yeast cells, while the normal structure of IRMCESH58L was revealed by the scanning electron micrographs. SEM analysis showed that the crushed phenotype of *C. auris* cells among the IRMCESH58L-induced *C. auris* cell regions was significantly reduced after the treatment compared to T3 (Figure 5).

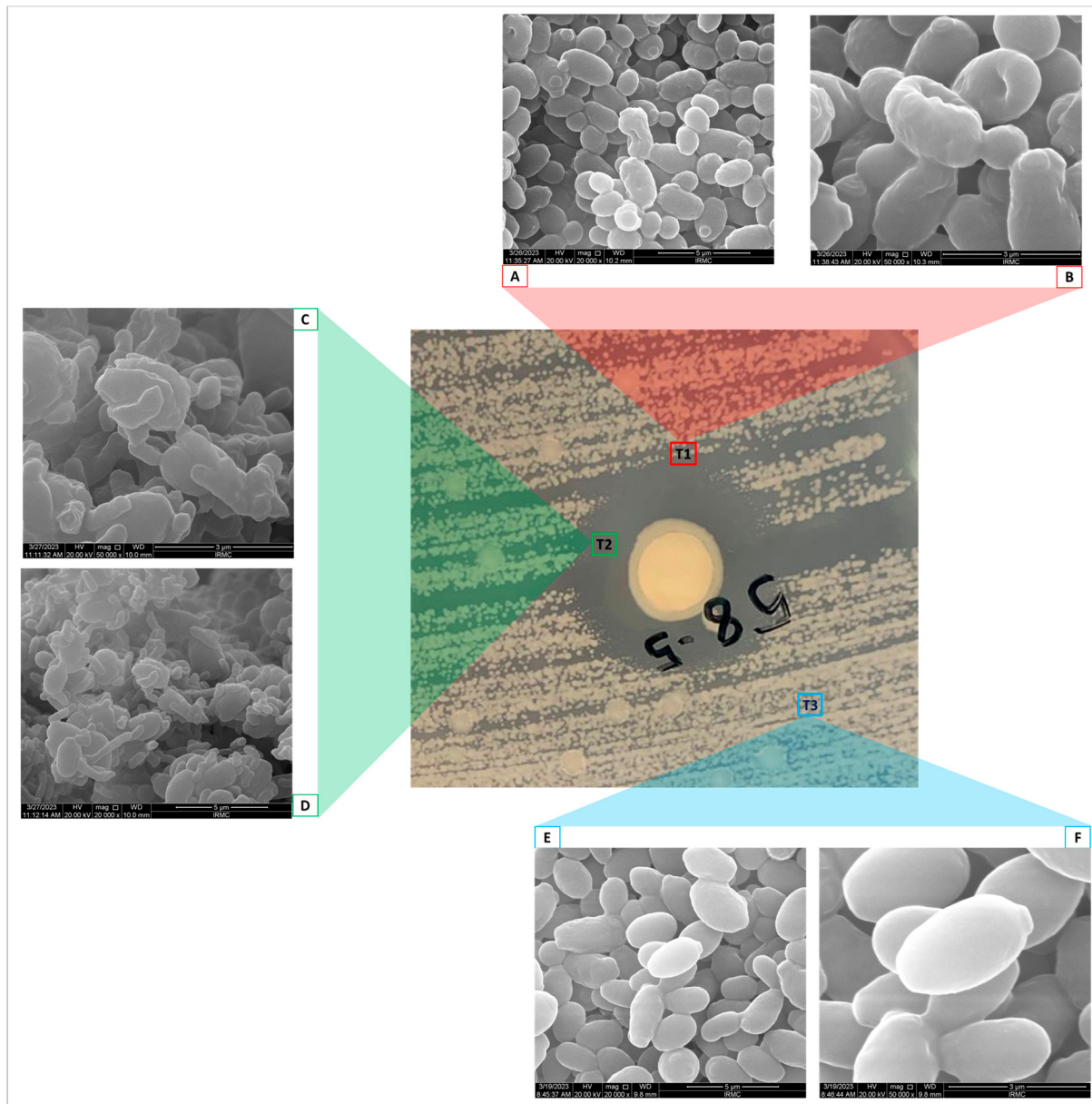


Figure 5. Morphological impact of IRMCESH58L on *Candidozyma auris* using SEM analysis. Electron microscopic structure of *C. auris* and IRMCESH58L after antifungal susceptibility of IRMCESH58L to *C. auris*. (A,B) Electron microscopic structure of *C. auris* from the edge of the zone of inhibition [T1]. (C,D) *C. auris* cell membranes disrupted by the IRMCESH58L structure of *C. auris* from the zone of inhibition [T2]. (E,F) Electron microscopic structure of *C. auris* from the normal fungal growth region [T3]. Blastozoonidia of *C. auris* appear as oval or spherical cells.

3.4. 16S rRNA Gene Sequencing of IRMCESH58L

The genomic DNA was extracted successfully, and the amplified 16S rRNA gene was confirmed by gel electrophoresis for the ~1400 bp amplicon. The amplified 16S rRNA gene was purified and sequenced. The 16S rRNA gene of bacterial isolate IRMCESH58L was aligned with reference sequences from the genus *Vibrio* and closely related to *Vibrio alginolyticus* (Figure 6).

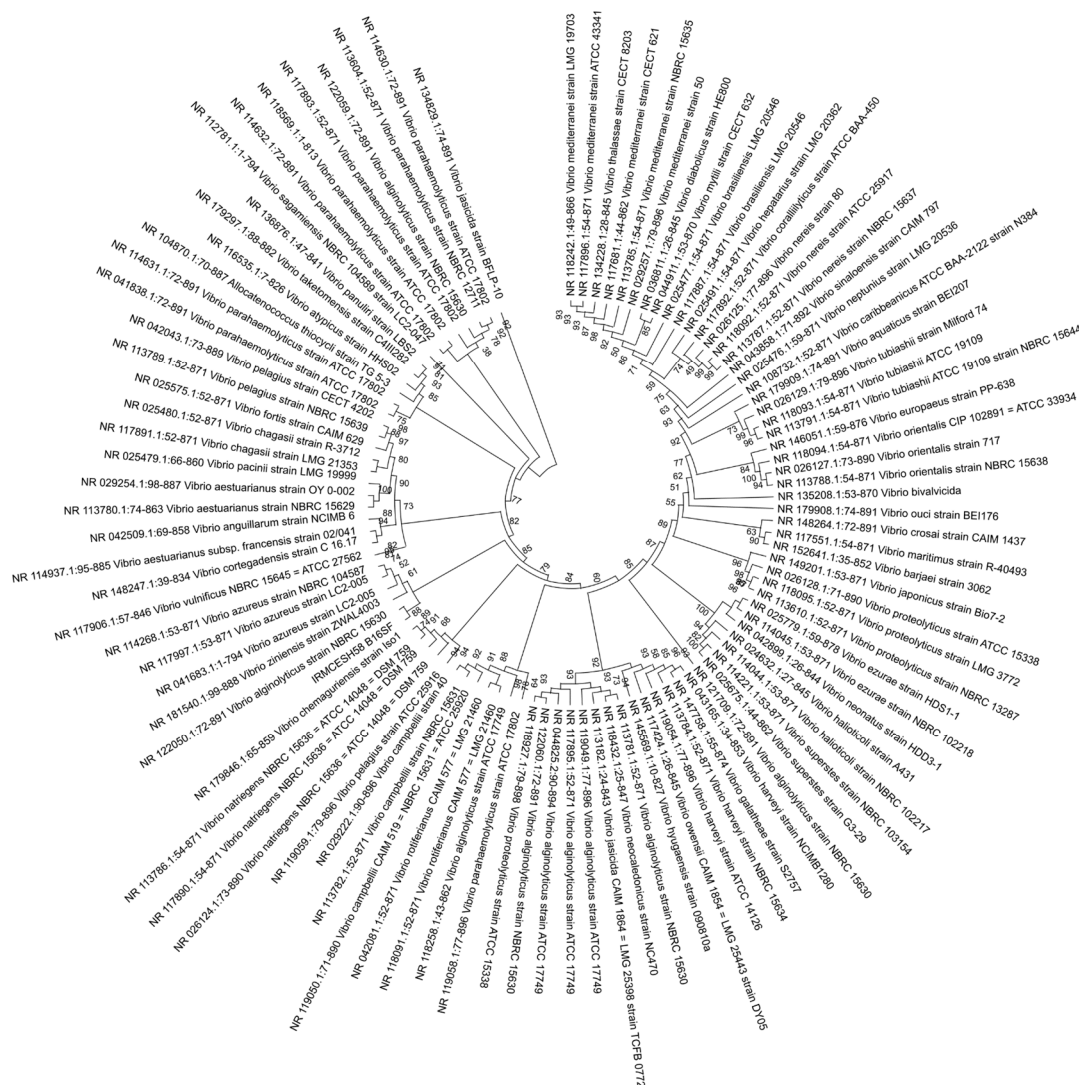


Figure 6. Molecular identification of IRMCESH58L using the 16S rRNA gene sequence and phylogenetic analysis.

3.5. Cell Cytotoxicity

The cytotoxicity effect of IRMCESH58L-cultured broth was evaluated via MTT assay on HFF-1 cells after incubating each control broth and IRMCESH58L-cultured broth at 50%, 40%, 20%, 10%, 5%, and 1% for 24 and 48 h. The diluents used showed high cell viability averages of between 86 and 102%, indicating that there is no cell cytotoxicity at 50% concentration. However, the cell viability plots of the control broth and IRMCESH58L-treated cells are correlated and show no significant differences (Figure 7). Light microscopy imaging of treated HFF-1 cells showed no difference in cell growth or morphology.

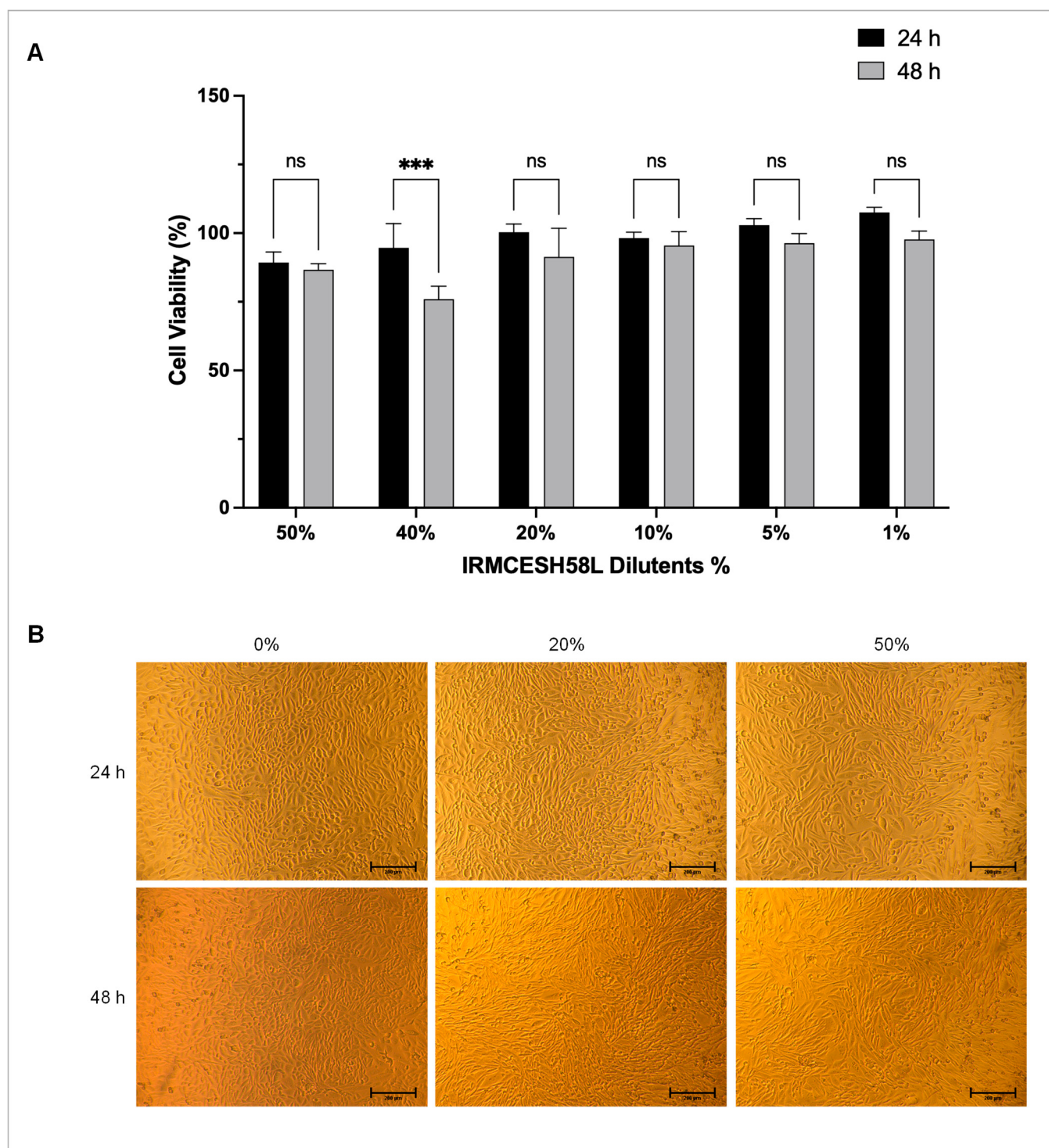


Figure 7. (A) Evaluation of the cell viability via MTT assay in HFF-1 cells. HFF-1 cells were treated with IRMCESH58L-cultured media diluents at 50%, 40%, 20%, 5%, and 1% for 24 and 48 h. The results were analyzed from three triplicates at each time point using two-way ANOVA with the Bonferroni multiple-comparison test. Significance is indicated at a p -value of <0.05 , and (ns) denotes non-significant differences ($p \geq 0.05$). (B) Microscopy images of IRMCESH58L-treated HFF-1 cells. Top: HFF-1 cells treated with RMCESH58L-cultured media diluents in DMEM at 20% and 50% for 24 h; bottom: for 48 h (magnification 100 $\times$; scale bar = 200 μ m). *** Significant at a p -value of <0.05 ; ns: non-significant.

3.6. IRMCESH58L Genome Analysis

The IRMCESH58L genome was sequenced using long reads from Oxford Nanopore. The genome assembly revealed that the IRMCESH58L genome has 6,556,025 bp, is plasmid-free, and has an average sequence length and G+C content of 48.93% (Table 1). The assembled IRMCESH58L genome contains 49 contigs, with contig N50 of 909,243. The IRMCESH58L genome has 11,226 protein coding sequences (CDS) predicted by the annotation (Table 2).

Table 1. Assembly details of *Vibrio* sp. IRMCESH58L genome.

S. No.	Assembly Details	IRMCESH58L Genome
1	Contigs	49
2	GC Content	48.93
3	Plasmids	0
4	Contig L50	3
5	Genome Length	6,556,025 bp
6	Contig N50	909,243

Table 2. Genome features of *Vibrio* sp. IRMCESH58L genome.

S. No.	Genome Features	IRMCESH58L Genome
1	CDS	11,226
2	tRNA	135
3	Repeat Regions	0
4	rRNA	33
5	Partial CDS	0
6	Miscellaneous RNA	0

The assembled *Vibrio* sp. IRMCESH58L genome was subjected to protein features, which revealed that the IRMCESH58L genome has 7883 proteins with functional assignments, and 1838 proteins with pathway assignments were identified (Table 3).

Table 3. Protein features in the genome of *Vibrio* sp. IRMCESH58L.

S. No.	Protein Features	IRMCESH58L Genome
1	Hypothetical proteins	3343
2	Proteins with functional assignments	7883
3	Proteins with EC number assignments	2533
4	Proteins with GO assignments	2148
5	Proteins with Pathway assignments	1838
6	Proteins with genus-specific family assignments	0
7	Proteins with cross-genus family assignments	9428

The strain *Vibrio* sp. IRMCESH58L is closely related to *Vibrio alginolyticus*, as they share a recent common ancestor and form a distinct, well-supported cluster with a high percentage of similarity in the branching (Figure 8). *Vibrio* sp. IRMCESH58L is positioned on a main branch and shows a high genetic relatedness to *Vibrio alginolyticus*, indicated by their close cluster, but it is also near *Vibrio diabolicus* and *Vibrio shiloi*. This placement suggests it is a marine bacterium. The genomic map of *Vibrio* sp. IRMCESH58L has a large cluster of ORFs, labeled VF_0841 to VF_0860, located near the origin (0 Mbp/6.5 Mbp) and spanning the dark blue segment (Figure 9). This specific segment represents a biosynthetic

contains a cluster of genes, including Glyoxylase family protein and death-on-curing protein, which appears to be present in *Vibrio* sp. IRMCESH58L and absent in other strains. This suggests differences in their potential metabolic and survival pathways.

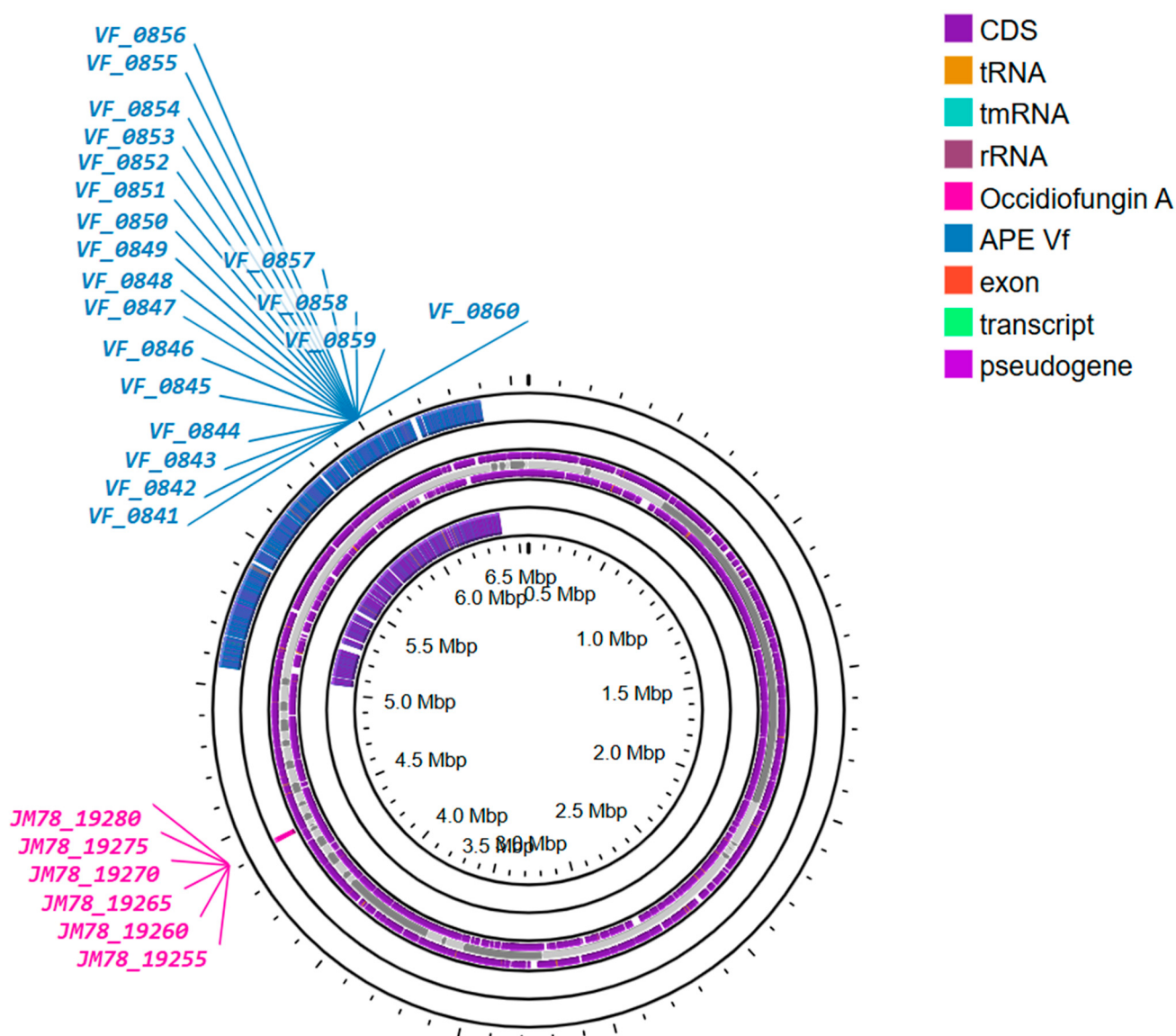


Figure 9. Known BGC codes for surfactin located on the whole-genomic map of *Vibrio* sp. IRMCESH58L.

3.7. LC-MS Analysis

To demonstrate the production of biosurfactants by *Vibrio* sp. IRMCESH58L, comprehensive LC-MS analysis was performed in both positive and negative electrospray ionization (ESI) modes (Figure 11). The resulting spectra provide robust analytical evidence of surfactin-like lipopeptide production. In the positive mode, we identified a characteristic series of ions with mass-to-charge ratios (m/z) of 591.38, 635.41, 679.43, and 723.46, representing different congeners or fragments of the lipopeptide complex. Additionally, the negative mode revealed prominent peaks at m/z 365.24 and 393.27, alongside fatty acid-related signals at m/z 255.22 and 281.24. These findings move the identification beyond speculation, confirming that IRMCESH58L produces bioactive lipopeptides consistent with the surfactin family, a trait that likely contributes to its antifungal activity against *C. auris*.

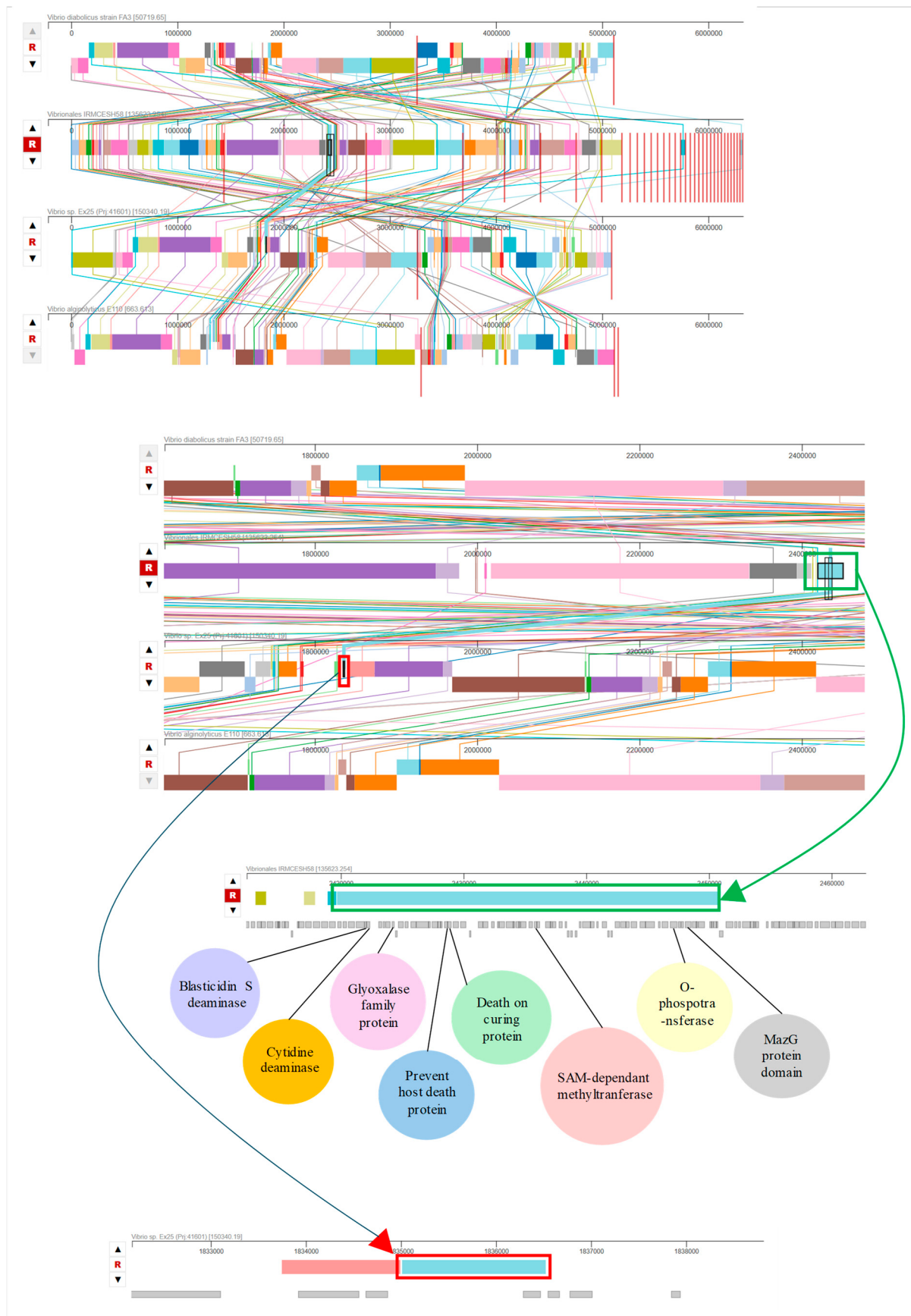


Figure 10. Whole-genome comparison of *Vibrio diabolica*, *Vibrio alginolyticus*, *Vibrio* sp. Ex25, and *Vibrio* sp. IRMCESH58L.

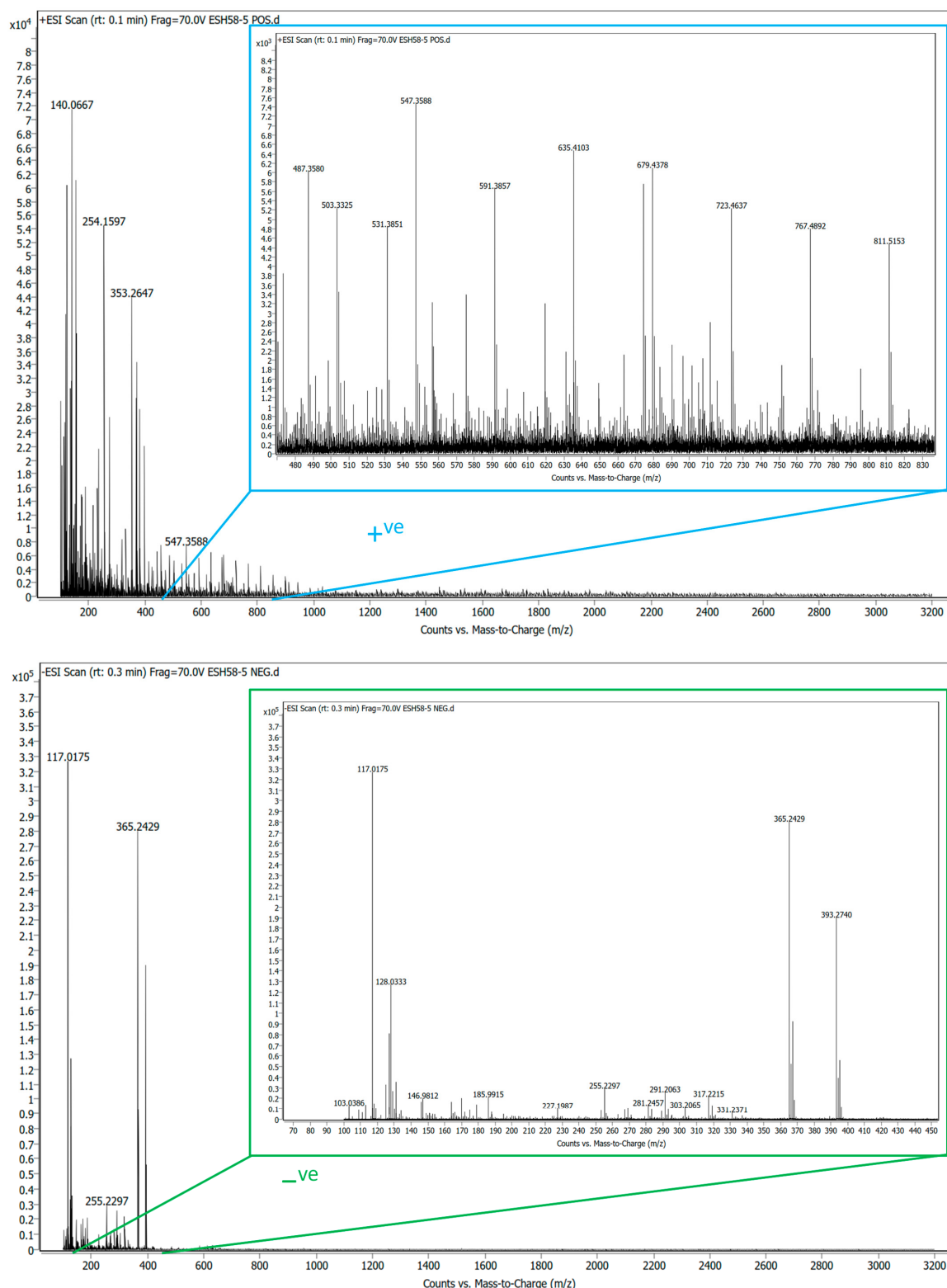


Figure 11. LC-MS analysis of *Vibrio* sp. Irmcesh58L. LC-MS characterization of the bioactive extract from *Vibrio* sp. Irmcesh58L. **(Top)** Positive ESI-mode scan revealing a series of peaks separated by approximately 44 Da (m/z 591.38, 635.41, 679.43, 723.46, 767.48, and 811.51). This periodic distribution is indicative of a lipopeptide complex, such as the surfactin family, containing varying lengths of hydroxy fatty acid chains. **(Bottom)** Negative ESI-mode scan showing prominent molecular ions at m/z 365.24 and 393.27, alongside characteristic fragments at m/z 117.01, 255.22, and 317.22. These peaks are consistent with the presence of deprotonated fatty acid chains and peptide moieties.

4. Discussion

We successfully used whole-genome sequencing to identify the isolated marine bacteria at the genomic level. Various samples collected from the marine region were used to isolate bacteria. The initial screening showed that three bacteria were effective against *C. auris* and three samples had activity as a consortium. However, they showed no anti-*C. auris* activity when separated. Hence, we have continued the screening to isolate individual colonies with anti-*C. auris* activity. We isolated a strain, IRMCESH58L, and identified it as *Vibrio alginolyticus* through 16S rRNA gene sequencing. The experiments detected that the possible antifungal metabolites of *Vibrio* sp. IRMCESH58L in the zone of inhibition damaged the cell wall membranes of *C. auris* at the edge of the zone and significantly reduced their size as a result of the treatment, as observed when examining the bacteria from the zone of inhibition via SEM. The results of this study have significant implications for understanding how IRMCESH58L affects *C. auris*. The isolated bacterial strain, IRMCESH58L, was further analyzed by long-read genome sequencing analysis. IRMCESH58L exhibits high genetic relatedness to *Vibrio alginolyticus*, but it is also positioned near *Vibrio diabolicus* and *Vibrio shiloi*; hence, the IRMCESH58L strain is considered as *Vibrio* sp. IRMCESH58L. IRMCESH58L was initially assigned to *V. alginolyticus* via 16S rRNA sequencing; however, following whole-genome sequencing, phylogenomic metrics suggested a broader classification. To maintain taxonomic accuracy, the isolate was designated as *Vibrio* sp. pending further detailed characterization. An experimental in vitro assessment of the toxicity of cell-free secondary metabolites of *Vibrio* sp. IRMCESH58L against HFF-1 cells was also conducted based on the MTT assay, and it clearly indicated no cytotoxicity. While HFF-1 cells showed no cytotoxicity in this study, we acknowledge the limitation of using a single cell line. To ensure accuracy, all assays were performed in triplicate, yielding reproducible data. Future studies will evaluate IRMCESH58L across diverse human cell types to further validate its safety profile and clinical potential.

In our study, we identified a secondary metabolite gene cluster by analyzing the whole-genome sequence of the bacterial isolate with anti-*C. auris* activity and detecting specific biosynthetic gene groups. The identification of this cluster is highly relevant as it may play a major role in the antifungal effects observed against *C. auris*. The genomic map of *Vibrio* sp. IRMCESH58L shows a large cluster of ORFs in a specific segment representing a biosynthetic gene cluster, which is coded for surfactin and confirmed on contig 6 of the genome, providing a genetic basis for the observed antifungal activity. A total of 64 samples were systematically collected from a coastal region of Eastern Saudi Arabia, encompassing diverse sources, including saltwater, seaweeds, rocks, and fish, across various locations. This sampling strategy was employed to ensure the isolation of a wide variety of culturable bacteria, which ultimately led to the selection of *Vibrio* sp. IRMCESH58L for whole-genome sequencing and further analysis [27–29]. Three samples of these marine bacterial strains, namely, IRMCESH58L, IRMCESH39, and IRMCESH42, showed antifungal activity against *C. auris* but were not persistent, except for the IRMCESH58L bacteria. It is challenging to extract and grow microorganisms from saltwater samples due to the high salinity, poor supply of nutrients, and temperature variability. Additionally, many marine microbes are selective, requiring specific nutrients or conditions for growth that cannot be found in regular laboratory media [30]. As a result, the success rate of identifying specific strains or types for study can be very low when using saltwater samples as a source of microorganisms. Earlier studies revealed the difficulty of isolating marine microorganisms from seawater. Only a few types of bacteria were successfully isolated from the deep South China Sea [31]. Lopatina et al., in another study, tried to collect samples from the Arctic Sea to identify bacteria; the authors found that they had a very low number of bacterial strains. The present study also successfully isolated only a few strains, but despite the dif-

difficulties, it was successful in identifying novel bacterial species with anti-*C. auris* activity, similar to an earlier study that reported many novel bacterial species and described their metabolic characteristics [32]. We were able to uncover several bacteria that produce antifungal compounds against *C. auris* by using culture techniques and specific growth media. Our research is compatible with previous studies that have shown how marine microorganisms [33,34] and bacteria from other sources could be used as a source of antibacterial and antifungal synthetic substances [35]. The findings suggest that marine bacteria, particularly *Vibrio* sp. IRMCESH58L, are a promising source of new antifungal compounds, and that mining the genomes of marine bacteria is a promising avenue for the development of new drugs to overcome multidrug resistance in fungal infections. This research highlights *Vibrio* sp. IRMCESH58L as a non-cytotoxic source of anti-*C. auris* metabolites, specifically coded with a surfactin gene cluster. While this provides a reliable baseline, the lack of dose- and time-dependent data is a limitation of the current study. To enhance the impact of this line of inquiry, in the future, focus must shift toward scaling metabolite purification and assessing potential host toxicity and the unavoidable risk of *C. auris* developing resistance to these natural compounds.

A previous study focused on mining the genome of marine bacteria to find gene clusters involved in the secondary metabolites that generate bioactive compounds against *Candidozyma auris*, but there have been no promising findings against *C. auris* infection [36], barring a few studies from the current research team [35,37,38]. The goal of this study was to determine how the properties of bacteria affect how efficiently drugs work against *C. auris* strains and to identify potential drug molecules that could work against the fungus, which is resistant to many drugs. The anti-*C. auris* activity of IRMCESH58L motivated the investigation of the genes responsible for encoding natural antifungal compounds through genome mining, with the goal of discovering drug molecules effective against multidrug-resistant *C. auris*. The results of this study demonstrate the unique potential of *Vibrio* sp. IRMCESH58L for drug production against *C. auris* strains. This study is in line with recent reports that revealed the possible exploration of anti-*C. auris* compounds coded in the genome of nanotube-forming *Bacillus amyloliquefaciens* MR14M3, *Bacillus* sp. strain IRMC27M2, and *Bacillus halotolerans* AQ11M9 [35,37,38]. This observation holds promise for the development of novel drug components. A recent study by Borgio and colleagues (2023) identified a bacterial strain with anti-*Candida* activity that was isolated from Saudi Arabia and identified using 16S rRNA gene sequencing. The isolate, *Bacillus* MR14M3, was found to inhibit *C. auris*, and its 16S rRNA gene sequence aligned with *Bacillus amyloliquefaciens*. The strain's cytotoxicity and pathogenicity were also observed to be non-toxic and non-pathogenic. The study suggests using *Bacillus* MR14M3 as a biofactory for an anti-*C. auris* compound synthesizer due to its highly conserved biosynthesis-related gene clusters and anti-*C. auris* potentials. However, to our knowledge, no previous studies have reported the anti-*C. auris* activity of *Vibrio* sp. against *C. auris*.

The presence of this gene cluster in *Vibrio* sp. IRMCESH58L (shared with *V. diabollicus*) suggests that this strain possesses distinct metabolic and survival pathways that are lacking in the other two strains considered. This includes specialized functions for detoxification and, potentially, a mechanism [Glyoxylase family protein and a toxin–antitoxin (TA) system (death-on-curing protein and prevent-host-death protein)] for genetic stability or stress survival. As the first to report the anti-*C. auris* activity of *Vibrio* sp. IRMCESH58L in Saudi Arabia, our findings differ from those of previous studies that did not explore this phenomenon in *Vibrio* sp. The observation of anti-*Candida* activity in IRMCESH58L, its genome composition, and the presence of gene clusters encoding natural antifungal compounds highlight its significance in combating multidrug-resistant *C. auris* infections. Further in-depth studies are needed to confirm the subspecies status of *Vibrio* sp. IRM-

CESH58L and to fully exploit its potential as a novel drug candidate. Further pre-clinical trials of specific secondary metabolites from *Vibrio* sp. IRMCESH58L can reveal its complete exploitation against *C. auris* infection. To our knowledge, although there have been many studies on marine screening investigating bacteria against fungi, this study is the first to report bacteria with antifungal activity in Saudi Arabia and bacterial secondary metabolites against *C. auris*. Our research showed that native microorganisms from the marine region might be a source of novel medications for treating multidrug-resistant fungal diseases, especially candidiasis by *C. auris*. One limitation of our study is the difficulty of isolating bacteria from seawater. However, we were able to isolate a bacterium with antifungal activity against *C. auris*. It is possible that other marine bacteria with more effective antifungal compounds were undetected due to the number of isolated bacteria in the samples examined. It is also important to note that natural compounds may have limitations in terms of availability and scalability for large-scale production. Further investigations are required to address these limitations and validate the clinical potential of marine bacteria as a source of new drugs against *C. auris* and other multidrug-resistant fungal infections.

5. Conclusions

In this study, a novel *Vibrio* sp. IRMCESH58L bacterium was successfully isolated and characterized from the marine environment of Eastern Saudi Arabia, addressing the critical need for new therapies against the multidrug-resistant fungal pathogen *C. auris*. We isolated 64 bacterial strains from diverse marine samples and identified the strain from fish liver as that with the highest and most persistent anti-*C. auris* activity. Molecular identification using *16S rRNA* gene sequencing and phylogenomic analysis identified this isolate as *Vibrio* sp. IRMCESH58L, showing a close relationship to *Vibrio alginolyticus*. Significantly, scanning electron microscopy analysis confirmed that the metabolites of *Vibrio* sp. IRMCESH58L physically disrupted the cell membranes and lysed *C. auris* cells, providing direct evidence of the strain's fungicidal mechanism. Long-read whole-genome sequencing revealed a complex *Vibrio* sp. IRMCESH58L genome, as well as the presence of a surfactin biosynthetic gene cluster, providing a robust genetic basis for the observed anti-*C. auris* activity. Furthermore, the cell-free culture broth confirmed no cytotoxicity against HFF-1 human cells, highlighting a favorable therapeutic profile. As the first report of the anti-*C. auris* activity of *Vibrio* sp. in the Arabian marine region, our study firmly establishes *Vibrio* sp. IRMCESH58L as a promising and novel bio-resource for the discovery and development of new antifungal drug candidates, offering a potential path to overcoming the global challenge of multidrug-resistant *C. auris* infections.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pharmaceutics18020266/s1>, Table S1: Characteristics of the isolated bacterial isolates from marine resources.

Author Contributions: Conceptualization: E.S.A., S.A.A., J.F.B. and N.B.A.; Methodology: E.S.A., S.A.A., J.F.B., N.B.A., R.A. (Reem AlJindan), N.M., S.A., D.A., H.H.A., S.H., R.A. (Rahaf Alquwaie) and T.S.D.; Software: E.S.A., S.A.A., J.F.B., N.B.A., S.A., D.A., H.H.A., S.H., R.A. (Rahaf Alquwaie) and T.S.D.; Validation: E.S.A., S.A.A., J.F.B., N.B.A., R.A. (Reem AlJindan), N.M., S.A., D.A., H.H.A., S.H., R.A. (Rahaf Alquwaie) and T.S.D.; Formal analysis: E.S.A., S.A.A., J.F.B., N.B.A., R.A. (Reem AlJindan), N.M., S.A., D.A., H.H.A., S.H., R.A. (Rahaf Alquwaie) and T.S.D.; Investigation: E.S.A., S.A.A., J.F.B., N.B.A., R.A. (Reem AlJindan), N.M., S.A., D.A., H.H.A., S.H., R.A. (Rahaf Alquwaie) and T.S.D.; Resources: E.S.A., S.A.A., J.F.B., N.B.A., R.A. (Reem AlJindan), N.M., S.A. and D.A.; Data curation: E.S.A., S.A.A., J.F.B., N.B.A., H.H.A. and S.H.; Writing—original draft preparation: E.S.A., S.A.A., J.F.B., N.B.A., S.A., D.A., H.H.A. and S.H.; Writing—review and editing: E.S.A., S.A.A., J.F.B., N.B.A., R.A. (Reem AlJindan), N.M., S.A., D.A., H.H.A., S.H., R.A. (Rahaf Alquwaie) and T.S.D.; Visualization: E.S.A., S.A.A., J.F.B., N.B.A., R.A. (Reem AlJindan), N.M., S.A., D.A., H.H.A., S.H.,

R.A. (Rahaf Alquwaie) and T.S.D.; Supervision: S.A.A., J.F.B., N.B.A. and R.A. (Reem AlJindan); Project administration: S.A.A., J.F.B., N.B.A. and R.A. (Reem AlJindan); Funding acquisition: E.S.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

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

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