

Characterization of a Potentially Novel Antibiotic-Producing Strain JPT8 from Urban Worm Farm Soil

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ABSTRACT

Antimicrobial resistance (AMR) poses an escalating global health crisis, with few novel structural classes of antibiotics approved in decades. To address this gap, we isolated and characterized a potentially novel antibiotic-producing bacterium, strain JPT8, from urban worm farm soil – an underexplored niche enriched by organic inputs and anthropogenic selective pressures – using a multidisciplinary pipeline combining microbiology, biochemistry, and molecular biology.

Serial dilution and plating yielded over 500 colonies. Four isolates showed reproducible inhibition against *Staphylococcus aureus* in patch-on-lawn assays, with JPT8 producing the largest zones (up to 23 mm). Scale-up fermentation in minimal media, dichloromethane extraction, and bioactivity-guided fractionation via thin-layer and silica gel column chromatography identified stable bioactive compounds in fractions S7 and S10 (10–20 mm zones in disc diffusion). Morphological (circular, undulate, tan colonies), biochemical (Gram-positive, catalase-positive, endospore-forming), and 16S rRNA gene sequencing (forward read: 1129 bp raw, trimmed to high-confidence 616 bp alignment with 88% identity) revealed only 82% pairwise similarity to the closest match (*Bacillus pumilus* NJ-M2, NZ_CP012329.1) and 79% to other *Bacillus* references, falling well below standard species delineation thresholds (~98.7%).

These findings suggest that JPT8 represents a divergent lineage with antimicrobial potential; however, further genomic and chemical characterization is required.

Keywords: AMR, *Bacillus*, bioactive compounds, 16S rRNA sequencing, PCR, urban worm farm soil, vermicompost, microbiome.

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INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most pressing global health threats, rendering one in six bacterial infections resistant worldwide (1). This trend, driven by overuse in clinical, agricultural, and environmental settings, has compromised frontline treatments for common infections and increased mortality risks from multidrug-resistant bacterial infections, including both Gram-positive and Gram-negative pathogens. Despite the urgency, antibiotic discovery pipelines have largely stalled: no new structural classes have reached clinical use in over three decades, and most recent approvals are derivatives of existing scaffolds that rapidly encounter resistance (2,3). The decline stems from high attrition rates in traditional screening from known microbial sources, low hit rates against resistant strains, and economic barriers to development. Soil microbiomes remain a rich source of bioactive natural products, although conventional sampling has yielded diminishing returns (4,5). Underexplored niches, such as urban worm farm soils, may provide access to distinct microbial communities with antimicrobial potential (6,7).

This study aimed to isolate and characterize antibiotic-producing bacteria from urban worm farm soil. The environment was hypothesized to harbor microorganisms with bioactive potential against pathogens such as *Staphylococcus aureus*, which could be identified through an integrated microbiological, biochemical, and molecular pipeline. This approach led to the identification of strain JPT8, which exhibited potent activity against *S. aureus* and partial sequence novelty relative to known *Bacillus* species. Subsequent fractionation confirmed the presence of bioactive components, laying the foundation for advanced structural elucidation. These findings highlight urban soil microbiomes as an accessible, high-yield source for antibiotic discovery and demonstrate how streamlined multidisciplinary methods can revive natural product pipelines amid escalating AMR (8).

MATERIAL AND METHODS

The experimental workflow (Figure 1) began with sample collection and bacterial isolation, followed by primary antimicrobial screening using patch-on-lawn assays. After the potent isolate JPT8 was identified in the initial screening, two parallel workflows were pursued: (1) Scale-up fermentation, biomolecular extraction, and chromatographic fractionation for bioactivity guided purification of active compounds, and (2) morphological, biochemical, and molecular characterization of the strain.

REAGENTS AND MATERIALS

The reagents used in this study, along with their suppliers, were as follow: Trypticase soy agar (TSA), nutrient agar (NA), nutrient broth (NB), and Mueller-Hinton agar (MHA) from Carolina® Biological (Burlington, NC, USA); minimal media components including soluble starch, yeast extract, peptone from Carolina® Biological (Burlington, NC, USA); Dichloromethane, methanol, chloroform, diethyl ether, toluene, ethyl acetate, and Physiological saline (0.85% NaCl) from Fisher Scientific (Pittsburgh, PA, USA) and Acros Organics (New Jersey, USA); DNeasy Blood and Tissue Kit for DNA extraction from Qiagen (Hilden, Germany); universal 16S rRNA primers (27F and 1492R) – Integrated DNA Technologies (San Diego, CA, USA); silica plates (Whatman 4410222); and sterile 6 mm disc (Becton Dickinson BBL, Sparks, MD, USA).

SAMPLE COLLECTION AND BACTERIAL ISOLATION

Soil samples were collected from an urban worm farm at 10–20 cm depth using a sterile borer in December 2014 and stored at 4°C. One (1) gram of soil was suspended in 9 mL sterile physiological saline (Fisher Scientific), homogenized by vortexing for 1 min, and serially diluted to 10^2 , 10^3 , 10^4 , 10^5 , and 10^6 concentrations. Aliquots (0.1 mL) were spread on trypticase soy agar (TSA; Carolina 788428) and incubated at 28°C for 3 days to achieve proper growth. Distinct colonies were purified by repeated streaking on nutrient agar (NA; Carolina 785301) at 28°C for 24 h and preserved at 4°C for further experimentation.

ANTIMICROBIAL SCREENING

Physiologically distinct microbial populations were selected for study of their antimicrobial properties. Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) were activated in nutrient broth (NB Carolina 785361) for 18 h at 37°C before careening. Primary screening used patch-on-lawn assays on Mueller-Hinton agar (MHA; Carolina 784942) at 37°C for 24 h. The patches were placed on fresh lawn cultures of bacterial strains, and the plates were incubated at 37°C for 24 h. The secondary screening of extracted biomolecules employed the Kirby-Bauer disc diffusion assay. A known amount of extract was absorbed onto 6-mm sterile discs (BD, BBL), and the discs were placed on fresh lawn growth of test organisms on MHA media. All experimental plates were incubated at 37°C for 24 h. To ensure the accuracy and objectivity of inhibition zone measurements, a standardized protocol was followed. All zones were measured manually using a calibrated metric ruler against a high-con-

trast background to the nearest 0.1 mm. For disc and patch, two perpendicular diameters were measured, and the average value was recorded.

SCALE-UP AND BIOMOLECULE EXTRACTION

Potent culture - JPT8 was selected for mass culturing using lab scale fermentation. A well-isolated colony was inoculated in minimal media containing 1% starch, 0.4% yeast extract, 0.2% peptone and incubated for 7 days with shaking. The biomass was harvested by centrifugation, and metabolites were extracted with an equal volume of dichloromethane (DCM) in duplicate. Extracts were evaporated under reduced pressure using a rotary evaporator (Buchi), air-dried, and collected as vial 1 and vial 2, respectively. The dried extract was resuspended in dichloromethane to a concentration of 10 mg/mL and absorbed onto sterile 6 mm disc (BD, BBL) for disc diffusion testing.

CHROMATOGRAPHIC FRACTIONATION

Crude extracts were analyzed by thin-layer chromatography (TLC) on silica plates (Whatman 4410222) developed in methanol: chloroform (9:1) and visualized under UV light. Preparative fractionation used silica gel column chromatography with diethyl ether: toluene gradient ratio: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. Ten fractions (S1 – S10) were collected in sterile screw cap vials, evaporated, and screened for antimicrobial activity.

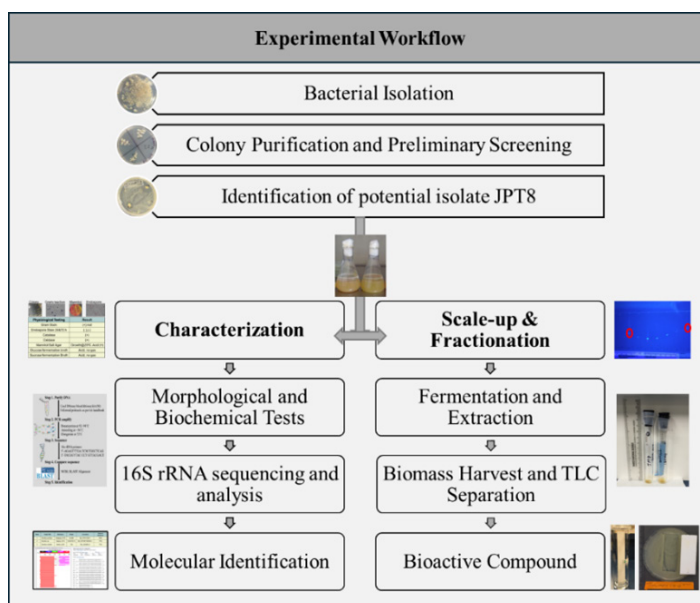
PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF STRAIN JPT8

Strain JPT8 was characterized phenotypically by colony morphology on nutrient agar, Gram staining, endospore formation, catalase and oxidase tests, and sugar fermentation patterns according to established protocols (9).

For molecular characterization, JPT8 was cultured in minimal broth for 72 h and harvested by centrifugation at 5000 rpm for 10 min. The supernatant was discarded, and the cell pellets were resuspended in 180 µL of enzymatic lysis buffer, then incubated at 37°C for 30 min. Subsequently, 25 µL proteinase K and 25 µL Buffer AL were added, mixed by vortexing, and incubated at 56°C for 30 min. Genomic DNA was purified using the DNeasy Blood and Tissue Kit (Qia-gen) according to the manufacturer's protocol. The lysate was applied to a DNeasy spin column and centrifuged at 8000 rpm for 1 min. The column was washed sequentially with 500 µL Buffer AW1 and 500 µL Buffer AW2, followed by centrifugation at 8000 rpm for 1 min and 20,000 rpm for 3 min, respectively.

FIGURE 1.

Workflow for isolation, characterization, and bioactivity-guided analysis of strain JPT8.



The schematic illustrates bacterial isolation and preliminary screening, followed by parallel scaleup pathways for strain characterization (morphological, biochemical, and 16S rRNA analysis) and fermentationbased extraction with chromatographic separation of bioactive compounds.

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The resulting 16S rRNA gene amplicon from strain JPT8 was sequenced in the forward direction only using primer 27F. The resulting Sanger read (raw length 1129 bp, containing 360 ambiguous 'N' bases, which reduced the usable high-quality sequence length) was quality-trimmed (Phred ≥ 20) and analyzed using BLASTN against the NCBI nucleotide database.

All experiments were performed in triplicate. Results are reported as mean $\pm$ standard deviation ($n=3$). No statistical comparisons between groups were performed.

RESULTS

ISOLATION OF ANTIBIOTIC-PRODUCING STRAINS

Serial dilution and plating on trypticase soy agar yielded over 500 colonies after 3 days at 28°C. Eleven morphologically distinct isolates were purified by repeated streaking on nutrient agar. Patch-on-lawn assays against *Staphylococcus*

aureus identified four strains, whereas *Escherichia coli* showed no recordable zone of inhibition. Strain JPT8 produced the largest inhibition zones (22.6 ± 1.06 mm, $n = 3$) in initial patch assays and was selected for further characterization as the most promising candidate (Figure 2a/2b).

ANTIMICROBIAL ACTIVITY AND BIOMOLECULE FRACTIONATION

Scale-up fermentation of JPT8 produced crude dichloromethane extracts with consistent activity against *S. aureus* yielding a mean inhibition zone of 17.4 ± 2.12 mm (Vial 1) and 23.5 ± 1.17 mm (Vial 2) ($n = 3$) in disc diffusion assays (Figure 2c). Thin-layer chromatography (TLC) of crude extracts in Methanol: Chloroform (9:1) resolved four UV-visible bands, indicating chemical complexity. The extract was separated into 10 fractions by silica gel chromatography. Fractions S7 and S10 retained strong antimicrobial activity with mean inhibition zone 10.4 ± 0.72 mm and 19.5 ± 0.70 mm ($n = 3$), respectively (Figure 2d). These results suggest that JPT8 produces one or more bioactive compounds with stronger activity against *S. aureus* than against *E. coli* amenable to bioactivity-guided fractionation.

MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF STRAIN JPT8

Morphological and biochemical tests indicated JPT8 as a Gram-positive, endospore-forming rod. Colonies on nutrient agar were circular, approximately 2 mm in diameter, with undulate margins, opaque texture, dryness, and tan coloration. The strain was catalase-positive, oxidase-negative, and

fermented sugars. These traits are consistent with the genus *Bacillus*.

BLASTN analysis yielded a local alignment of 616 bp with 88% identity (541/616 matches, 5 gaps) to the 16S rRNA gene region in the *Bacillus pumilus* NJ-M2 genome (NZ_CP012329.1, positions 91033–91646). Overall pairwise similarity across the query was 82% to *Bacillus pumilus* NJ-M2, 79% to *Bacillus* sp. EGD-AK10, and 79% to *Bacillus subtilis* 168. This local alignment length reflects the high-quality, trimmed portion of the forward Sanger read after removal of low-confidence bases and ambiguities (N's). This phylogenetic positioning is illustrated in Figure 3.

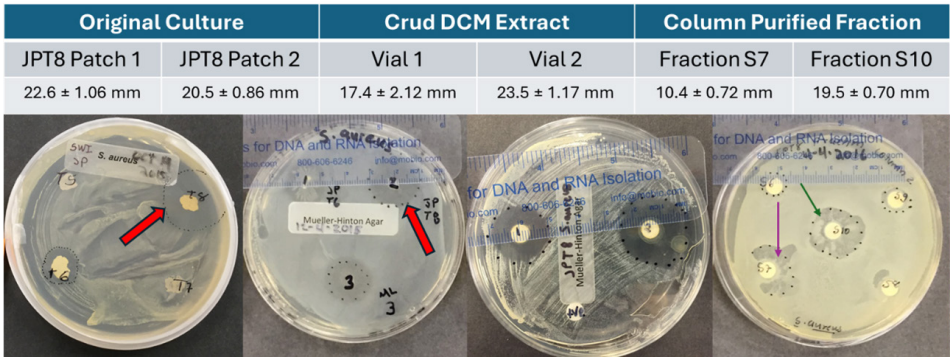
DISCUSSION

The isolation of strain JPT8 from urban worm farm soil yielded a Gram-positive bacterium with consistent antimicrobial activity against *Staphylococcus aureus* but none against *Escherichia coli*. This Gram-positive specificity is likely due to the outer membrane barrier in Gram-negative bacteria, which restricts access of the non-polar bioactive compounds to intracellular targets. Such selectively could be advantageous for targeting therapies against pathogens such as methicillin-resistant *S. aureus* (MRSA) without broadly disrupting commensal Gram-negative microbiota.

Morphological and biochemical tests confirmed traits consistent with the genus *Bacillus*. However, 16S rRNA sequencing provides overall pairwise similarities well below ~98.7% threshold typically required for species delineation and indicates phylogenetic divergence within the genus. Whole-genome sequencing or multi-locus sequencing will be

FIGURE 2.

Antimicrobial efficacy of strain JPT8: Antimicrobial efficacy of strain JPT8 against Staphylococcus aureus.



This multi-panel figure demonstrates the inhibitory activity of JPT8 extracts using patch-on-lawn and disc diffusion assays. All values are mean $\pm$ standard deviation from triplicate measurements ($n=3$). (Panels A–D correspond to original patch, purified colony, crude extract Vial 1/2, and purified fractions S7/S10, respectively.)

necessary to confirm whether JPT8 represents a novel species or a highly divergent lineage.

Bioactivity-guided fractionation via TLC and silica gel column chromatography produced stable active fractions (S7 and S10) that retained strong antimicrobial activity against *S. aureus*. A notable limitation of the extraction protocol is the exclusive use of dichloromethane, a non-polar solvent that preferentially recovers lipophilic compounds. Because no parallel aqueous or methanolic extractions were performed, highly polar bioactive metabolites may have been missed. Antimicrobial activity was limited to two model organisms (*S. aureus* and *E. coli*) which restricting insights into the full antimicrobial spectrum and the mechanism of action.

Urban worm farm (vermicompost) soil represents an underexplored anthropogenic niche that may favor microbes with competitive bioactive traits. The low-resource, multidisciplinary pipeline demonstrated here offers a scalable model suitable for an educational setting.

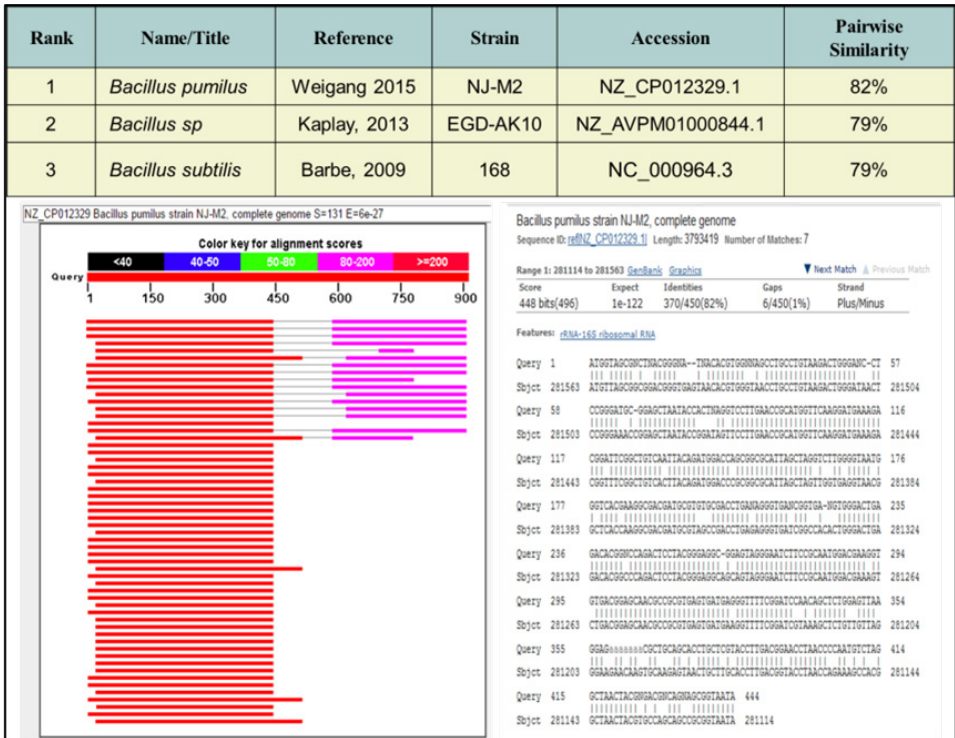
CONCLUSION

In summary, strain JPT8 isolated from urban worm farm soil demonstrated reproducible Gram-positive-specific antimicrobial activity against *Staphylococcus aureus* (zone of inhibition 17–23 mm) and 16S rRNA sequencing divergence from known *Bacillus* species (82% pairwise similarity). These findings supported by microbiology, biochemistry, and bioactivity-guided fractionation data, position JPT8 as a potential lead for further antibiotic discovery.

The multidisciplinary integration proved to be an effective, scalable approach to low-cost and accessible methods. Future work should focus on whole-genome sequencing of JPT8 to resolve its taxonomic position and identify biosynthetic gene clusters, structural elucidation of the active fractions (S7 and S10) using LC-MS/NMR, and expanded antimicrobial testing against clinically relevant resistant pathogens, including MRSA. These steps will determine the true potential of this early-stage discovery in contributing to the fight against antimicrobial resistance.

FIGURE 3.

16S rRNA-based similarity of strain JPT8 to closest *Bacillus* relatives. JPT8 shows 82% pairwise similarity to *B. pumilus* NJ-M2 (NZ_CP012329.1), 79% to *Bacillus* sp. EGD-AK10 (NZ_AVPM01000844.1), and 79% to *B. subtilis* 168 (NC_000964.3).



These results, combined with the morphological, biochemical, molecular identity, and Gram-positive-species specific anti-microbial activity, support the interpretation of substantial phylogenetic divergence and potential taxonomic as well as antimicrobial novelty for JPT8.

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