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Broad-spectrum antibacterial properties of a *Pseudomonas* field isolate and potential rhizomicrobiome implications

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ABSTRACT

Plant growth promoting bacteria (PGPB) such as *Pseudomonas* have been widely acclaimed for their roles in sustainable food production and consumption of pollutants in contaminated soils. Yet, we still have much to learn about how dynamics of highly diverse microbial communities are influenced by PGPBs capable of antibacterial activity. This article characterizes the antimicrobial profile of an Indiana *Pseudomonas* field isolate. To our knowledge, this work is the first to demonstrate *Pseudomonas*-induced inhibition of the PGPB *Serratia marcescens*, as well as *Pseudomonas*-induced inhibition and/or color change of *Kocuria rhizophila*. These data raise intriguing questions about how best to maximize the efficacy of biofertilizers containing multiple different organisms while minimizing unintended disruption of soil microbiomes. In addition, similar to previously published *Pseudomonas* strains, our isolate inhibited growth of clinically relevant bacteria such as *Staphylococcus, Mycobacterium,* and *Sarcina*. Future studies thus should investigate whether *Pseudomonas*-derived antibiotics could have novel applications in treating opportunistic *Serratia, Kocuria,* or *Sarcina* human infections.

Introduction

In the past decade, research on plant growth promoting bacteria (PGPB) has provided a hopeful solution to the problem of feeding the expanding human population without artificial fertilizer-associated soil damage: using biofertilizers to shape and turbocharge crop-enhancing microbial mutualisms [1]. PGPBs boost plant growth by diverse mechanisms including enhancing nitrogen, phosphorous, and potassium availability, managing drought or salt stress, sequestering iron, contributing to plant hormone levels, and even decreasing pathogenic organisms' infection of plants [2-7]. Encouragingly, such benefits have been observed across a variety of crops [2, 8]. PGPB-based soil treatment has accordingly become big business, with the North American biofertilizer market accounting for $952 million in 2023 and projected to increase [9]. As the race for PGPB market shares accelerates, equitable access to crop enhancing techniques and microbes will be essential to combat climate change-associated exacerbations of already unconscionable hunger rates [10].

Despite the promise of biofertilizers, challenges remain. One complexifying factor is the ability of many PGPBs to produce antibacterial molecules. Soil amendments containing these organisms—though valuable for modifying rhizomicrobiomes (root bacteria) to enhance crop growth—also include the potential for unintended and possibly detrimental shifts in the balance of microbial organisms that associate with plant roots and help plants stay healthy. For example, common PGPBs like

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Pseudomonas and *Serratia* produce a number of secondary metabolites with antimicrobial activity [3, 11-14]. Understanding the antimicrobial properties of commonly used agricultural PGPB genera therefore will be key for optimizing biofertilizer soil amendment strategies, particularly in light of recent work emphasizing the importance of combinatorial PGPB applications to generate stable rhizobacterial communities that will continue to boost crop production even as environmental conditions change [7, 15]. The research objective for the experiments described in this manuscript was to characterize the antimicrobial profile of a *Pseudomonas* bacterium isolated from Indiana soil and thereby to further investigate potential inhibitory relationships among rhizomicrobiome organisms.

Materials and Methods

Initial isolation and 16S rRNA sequencing of the field isolates:

In an effort to identify organisms with novel antimicrobial properties, field samples were collected from locations on or near the Wabash college campus. The field isolate characterized in this manuscript was collected from the top 1 inch of topsoil near the western endzone of the recently constructed Wabash College football stadium in Crawfordsville, Indiana (40.0369° N, 86.9067° W). We thought this location would be interesting to sample since the soil had been undisturbed for a long time but then had been exposed to recent construction activities, perhaps putting unusual selective pressure on the soil bacteria. The soil sample was mixed with Luria broth (LB; a nutrient rich media used to culture bacteria), centrifuged in a microfuge at 1,000 rpm for 5 minutes to pellet debris, and used for mixed-organism inoculation of LB agar source plates prior to colony purification by isolation streaking on LB agar. Colonies were screened via cross streak analyses for antimicrobial production. After the initial screen, a single colony of the isolate described in this paper was grown in LB. The resulting culture was aliquoted to send for 16S rRNA sequencing by GENEWIZ (Azenta Life Sciences) using forward and reverse primers. Glycerol stocks of the same culture were prepared for long-term -80^{o}C storage of the organism.

To identify the species of the collected field isolate, we sequenced data over 1000+ nucleotides using the National Center for Biotechnology Information standard nucleotide blast analysis (Blastn) [16]. The *Pseudomonas* isolate described in this paper demonstrated greater than or equal to 98.95% 16S rRNA sequence similarity with a large number of type *Pseudomonas* (*P.*) strains including but not limited to *P. glycinae* (Accession MG692779.1), *P. kribbensis* (CP029608.1), *P. fitomaticsae* (CP075567.1), *P. gozinkensis* (CP062253.1), *P. allokribbensis* (CP062252.1), *P. serboccidentalis* (OP021715.1), *P. iranensis* (CP077092.1), *P. monsensis* (CP077087.1), *P. granadensis* (LT629778.1), *P. soyae* (NR_181891.1), *P. koreensis* (NR_025228.1), *P. reinekei* (NR_042541.1), and *P. moraviensis* (NR_043314.1). We thus are unable definitively to identify the Wabash isolate without full genome sequencing. However, using Gapped/PSI-BLAST methods [17], GENEWIZ identified *P. koreensis* strain Ps 9-14 (AF468452) as the closest 16S rRNA match to our strain [18]. Given the high 16S rRNA sequence similarity with multiple strains, we will use terms such as "Wabash *Pseudomonas* isolate" or "our field isolate" throughout this manuscript rather than a species identifier. These phrases are not intended as an official nomenclature proposal.

The initial isolation and sequencing of the Wabash *Pseudomonas* isolate and all experiments described in this manuscript were performed in accordance with the research review policies of Wabash College.

Commercially obtained organisms:

The following organisms were purchased from Carolina Biological Supply: Escherichia coli B (E. coli B; catalog #124300), Mycobacterium smegmatis (M. smegmatis; catalog #155180A), Pseudomonas fluorescens (P. fluorescens; catalog #155255), Sarcina aurantiaca (S. aurantiaca; catalog #155400), Serratia marcescens (S. marcescens: catalog #155452), Sporosarcina ureae (S. ureae; catalog #155518), and Staphylococcus epidermidis (S. epidermidis; catalog #155556A). Kocuria rhizophila (K. rhizophila) was purchased from WARD, catalog number 85W0966. Immediately after purchasing, organisms were grown under recommended media, aeration, and temperature conditions prior to freezing at -80°C as glycerol stocks. Glycerol stock samples then were used to inoculate fresh cultures for experiments.

Cross streak analyses:

Cross streak assays were used to test for antimicrobial activity of the *Pseudomonas* field isolate against multiple strains of bacteria simultaneously. Cross streak experiments were carried out using the procedure established by Carvajal [19]. Nutrient agar plates were horizontally streaked either with LB as a no-inhibition control or with the Wabash *Pseudomonas,* using 10 microliters (µl) spread across a 7 centimeter (cm) line in the center of each plate. Plates were incubated at room temperature (20-24°C) for 48 hours to allow diffusion of any potential antimicrobials from the *Pseudomonas* into the surrounding agar. After the 48 hours, four test organisms were pipetted vertically on

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each plate and grown at room temperature for 5 days before photographing. These organisms included *S. aurantiaca*, *K. rhizophila*, *S. ureae*, *M. smegmatis*, *S. marcescens*, *E. coli B*, and *P. fluorescens*. LB also was streaked vertically as a sterility control. Each vertical cross streak was from a 5µl culture pipetted across 2.5cm, beginning approximately 2 millimeters from the horizontal *Pseudomonas* or LB streak.

Because *Staphylococcus's* preferred growth is at 37°C, separate cross streak assays were performed to determine the impact of the Wabash *Pseudomonas* field isolate on *Staphylococcus epidermidis* growth at that temperature. Nutrient agar plates were horizontally streaked with the Wabash *Pseudomonas* isolate or LB (again using 10µl across 7cm in the center of each plate) and grown for 48 hours at room temperature before applying vertical streaks of *S. epidermidis* in triplicate per plate or LB. Plates were subsequently grown at 37°C for 7 days before photographing. As an additional control testing for agar nutrient depletion, we also ran triplicate assays testing whether *S. epidermidis* would inhibit its own growth under our cross streak conditions. For these assays *S. epidermidis* was streaked horizontally on nutrient agar and grown for 48 hours at 37°C before inoculating with LB or triplicate vertical streaks of *S. epidermidis,* and further incubating at 37°C for 7 days.

Inhibition of *M. smegmatis* by the Wabash *Pseudomonas* isolate was further characterized in a longer-term cross streak assay using LB agar. LB or Wabash *Pseudomonas* was horizontally streaked along a 7cm center line on LB agar and grown at room temperature for 48 hours. Each plate then was inoculated with a vertical streak of LB (top left of each plate) and three vertical streaks of *M. smegmatis* (top right of each plate, and two streaks under each horizontal streak) and grown at room temperature for 21 days.

Zonal inhibition assays:

Zonal inhibition assays were used as an additional method to test for the presence and strength of antimicrobial activity of the *Pseudomonas* field isolate. In these assays, the idea was to spread a test organism across a plate and then pipette a small amount of a different organism in the center; if the center organism produces an antimicrobial, the antimicrobial diffuses from the center and kills the surrounding test organisms, causing a "zone of inhibition." The concentration of the antimicrobial is highest nearest the central spotting location and becomes less concentrated as the molecule diffuses outward to the edge of the plate. In our assays, test organisms were spread onto agar plates, and then the center of the plate was immediately spotted with sterile broth or liquid cultures of actively growing Wabash *Pseudomonas* or *P. fluorescens*. Assays were carried out on the media recommended for each respective test organism: nutrient agar for *E. coli B, K. rhizophila, P. fluorescens, S. aurantiaca,* and *S. marcescens,* and LB agar for *M. smegmatis.* Plates were incubated at room temperature. The figure shows representative data from triplicate plates at day 7 for the nutrient agar samples and—because *M. smegmatis* grows more slowly than the other organisms—at day 8 for *M. smegmatis.*

Since some zones were not perfectly circular we recorded the zones of inhibition as a range, calculating both the minimum and maximum zone edge distance on replicate plates. The minimum zone distances were measured on triplicate plates as the millimeter radius of cleared area from the outermost edge of the center-spotted organism to the nearest colony morphologically consistent with the test organism. The minimum distances were averaged together. Similarly, the maximum zone distances were measured on the same triplicate plates as the millimeter radius of cleared area from the outermost edge of the center-spotted organism to the furthest edge of the cleared area. The average range of inhibition was considered to be the average minimum to average maximum inhibition.

To determine the stability of the observed color change in *K. rhizophila* in the presence of the Wabash *Pseudomonas* organism, representative plates of the *K. rhizophila* replicates were allowed to grow for 21 days, with photographs taken on days 7 and 21. In parallel we investigated whether other bacteria besides the Wabash *Pseudomonas* similarly induced a color change in *K. rhizophila*. For these assays, *K. rhizophila* was spread on nutrient agar plates prior to spotting immediately in the center with a liquid inoculant of broth, *E. coli B, K. rhizophila, P. fluorescens, S. aurantiaca, S. marcescens,* or *S. epidermidis* and growing at room temperature. Photos were taken on day 7 post inoculation and are representative of triplicate assays.

Growth curve assays:

To assess whether the Wabash *Pseudomonas* field isolate's antimicrobial is secreted and retains its antimicrobial capacity in extracellular supernatant apart from the living cells, we tested inhibition of test organisms by Wabash *Pseudomonas* cell free supernatant. The cell free supernatant was harvested from triplicate 40 hour 12ml LB cultures of Wabash *Pseudomonas* grown at room temperature (24-28°C) to an average Optical Density (OD_{600}) of 1.36 (standard deviation of 0.11) prior to 0.45um filtration. For each original triplicate Wabash *Pseudomonas* sample, the 12ml filtrate was divided into three 3ml portions. Each 3ml portion then was inoculated with one of the following test organisms: *S. aurantiaca, P. fluorescens,* or *E. coli B.* As a positive growth control

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we incubated 12ml triplicate cultures of LB for 40 hours at room temperature and then inoculated 3ml aliquots with *S. aurantiaca, P. fluorescens,* or *E. coli B*. Uninoculated LB was used as a parallel sterility control. Growth of the test organisms in either the Wabash *Pseudomonas* filtrate or in LB was measured by OD_{600} spectroscopy until the cultures grown in LB plateaued at an OD_{600} of 1.99.

Results

The Pseudomonas field isolate exhibits antimicrobial activity as assessed by cross streak assay

To characterize the *Pseudomonas* field isolate's antimicrobial profile, we first performed cross streak assays at room temperature, testing its inhibition of a variety of common soil and clinically relevant Biosafety Level 1 Gram positive bacteria (which have thick peptidoglycan cell walls), Gram negative bacteria (which have thin peptidoglycan cell walls with an additional cell membrane outside of the cell wall), and acid fast organisms (which have a waxy cell wall containing extensive amounts of glycolipids).

Our field isolate was allowed to grow on each plate for 48 hours to maximize diffusion of potential antimicrobials into the agar prior to applying the test organisms. We would expect this diffusion to result in the highest concentrations of antimicrobials being closest to the field isolate streak and decreasing as distance from the field isolate streak increases. The Wabash *Pseudomonas* isolate exhibited negative impacts on growth of *S. aurantiaca, K. rhizophila, M. smegmatis*, *S. marcescens,* and *E. coli B* (Fig. 1). Thus, our preliminary studies showed *Pseudomonas*-associated broad spectrum antimicrobial activity against representative Gram positive, Gram negative, and acid fast bacteria. However, our *Pseudomonas* did not substantively prevent growth of *S. ureae*, *P. fluorescens*, or itself (Fig. 1).

We also tested the impact of our isolate on *S. epidermidis*'s growth at *Staphylococcus's* preferred temperature of 37°C. At the warmer temperature, *S. epidermidis* grew on the LB agar control plate as expected but had zero visible growth in the presence of the Wabash *Pseudomonas* isolate (Fig. 1).

Since *Mycobacteria* are typically slow growing and can form robust biofilms over the course of a few weeks in lab culture, we were curious to determine whether the Wabash *Pseudomonas*'s inhibition of *M. smegmatis* observed in the initial cross streak assay (Fig. 1) continued through a longer time period and prevented *Mycobacterium* biofilm formation. We therefore ran extended-time cross-streak assays using *M. smegmatis* as the only test organism, vertically streaked in triplicate in equal volumes on

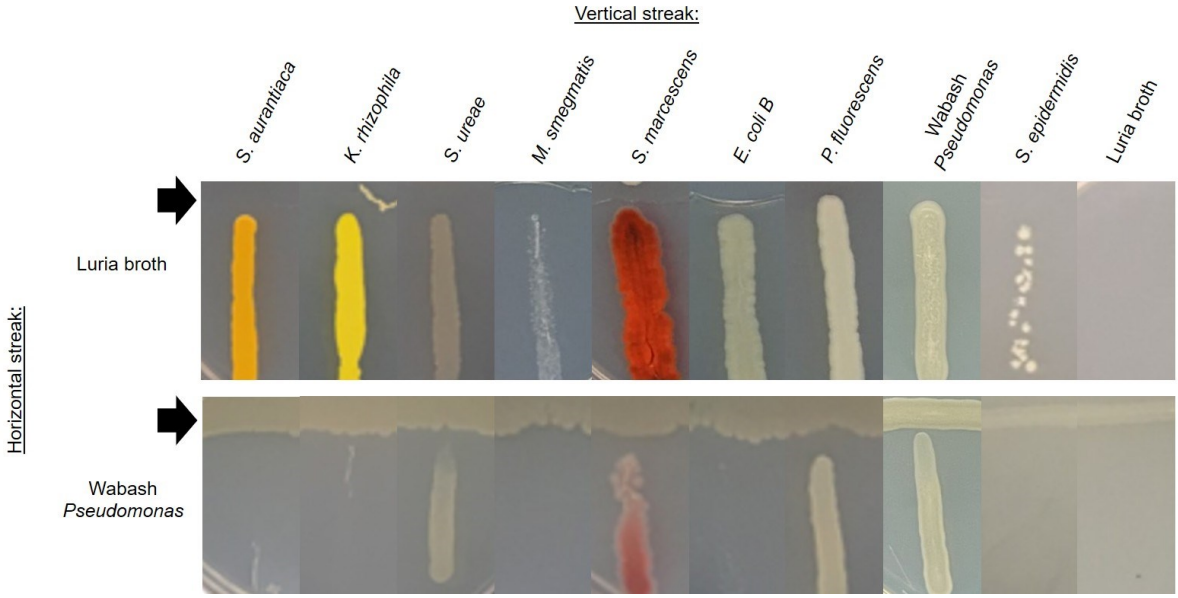

Fig. 1 Cross streak assay inhibition profiles of the *Pseudomonas* field isolate. The Wabash *Pseudomonas* field isolate and an LB control each were streaked horizontally on a nutrient agar plate and incubated at room temperature for 48 hours. Four test organisms then were pipetted vertically on each plate and grown at room temperature for 5 days before photographing. Gram positive test organisms: *S. aurantiaca*, *K. rhizophila*, and *S. ureae*. Gram negative test organisms: *S. marcescens*, *E. coli B*, *P. fluorescens,* and Wabash *Pseudomonas*. Acid fast organism: *M. smegmatis*. Vertical streaks of the Gram positive *S. epidermidis* were grown at *Staphylococcus's* preferred temperature of 37°C for 7 days. LB vertical streaks were included as a negative inhibition control.

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each plate along with a negative control LB-only streak (Fig. 2). By 3 weeks after adding the test organisms, *Mycobacterium* on the LB-streaked control plate was growing as a tan, flaky raised biofilm. Impressively, at the same time point there was no visible *Mycobacterium* growth on the plate containing the horizontal Wabash *Pseudomonas* streak.

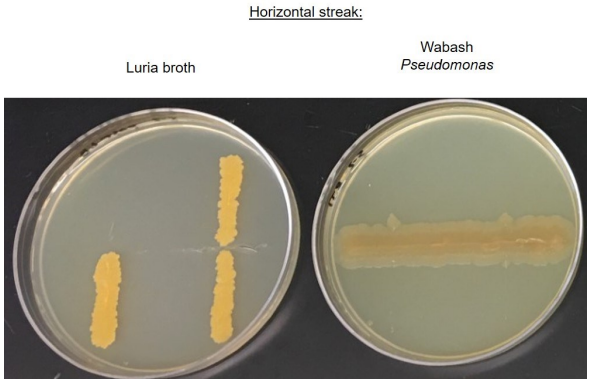

Fig. 2 Cross streak assay inhibition of *M. smegmatis* by the Wabash *Pseudomonas* isolate at room temperature by 3 weeks. Each plate was inoculated with a horizontal streak either of LB or the *Pseudomonas* field isolate as indicated and incubated at room temperature for 48 hours before adding a vertical streak of LB (top left of each plate) and three vertical streaks of *Mycobacterium smegmatis* (top right of each plate, and the two streaks under each horizontal streak).

Wabash Pseudomonas inhibition of Serratia, Sarcina, and Mycobacterium at room temperature is confirmed through zonal inhibition assays

Having demonstrated that several organisms were inhibited when the Wabash *Pseudomonas* was given a two day growth head start prior to applying the test organism (as in the Fig.1 and 2 cross streak assays), we next sought to determine if the field isolate could inhibit growth when inoculated onto an agar plate at the same time as the test organisms. This assay was intended to mimic an environmental soil condition where the *Pseudomonas* and test organism were given equivalent opportunity to grow, as opposed to the cross streak assay which gave the *Pseudomonas* an advantage. To this end we completed zonal inhibition assays for Wabash *Pseudomonas* using *E. coli B, S. marcescens, P. fluorescens, S. aurantiaca,* or *M. smegmatis* as test organisms. To examine whether the Wabash *Pseudomonas* strain's antimicrobial activity was unique or common to other *Pseudomonas* bacteria, we ran parallel zonal inhibition assays using the PGPB *P. fluorescens* as the center-spotting organism, measuring the nearest and farthest points of inhibition to determine the range of inhibition by either Wabash *Pseudomonas* or *P. fluorescens*. By one week after inoculation, the Wabash *Pseudomonas* isolate induced a large clear zone against *S. marcescens* (12.0 mm SD = 1.7 mm to 27.0 mm SD = 8.2 mm), *S. aurantiaca* (10.0 mm SD = 6.1 mm to 15.0 mm SD = 7.0 mm), and *M. smegmatis* (5.0 mm SD = 1.7 mm to 24.0 mm SD = 1.7 mm) (Fig. 3). There was a smaller zone of inhibition against *E. coli B* (0.0 mm SD = 0.0 mm to 6.7 mm SD = 5.9 mm) as well. Similar to our field isolate, *P. fluorescens* inhibited *S. marcescens* (2.7 mm SD = 3.8 mm to 18.0 mm SD = 12.5 mm) although the *P. fluorescens*-associated zone was smaller than that associated with the Wabash *Pseudomonas* (Fig. 3). Interestingly, however, *P. fluorescens* did not cause a zone of inhibition against the *S. aurantiaca* (0.0 mm SD = 0.0 mm to 6.4 mm SD = 10.4 mm) or *M. smegmatis* (0.0 mm SD = 0.0 mm to 12.7 mm SD = 3.8 mm) test bacteria and thus had a different antimicrobial profile than Wabash *Pseudomonas* (Fig. 3).

The antimicrobial activity of the Wabash Pseudomonos field isolate is retained in cell free supernatant.

The data in Figs.1-3 demonstrated antimicrobial activity of continuously growing agar cultures of the Wabash *Pseudomonas* field isolate but left open the question of whether the field isolate secreted a molecule whose antimicrobial activity would continue when separated from the producing colony. To answer this question we harvested cell-free supernatant from liquid cultures of the field isolate and inoculated them with test organisms, followed by growth curves measuring the replication of the test organism by spectrophotometric OD 600 assays. As proof of principle, we selected a Gram positive and a Gram negative test organism that had demonstrated full inhibition by cross streak assay (Fig. 1) and zonal inhibition (Fig. 3). To that end, we chose *S. aurantiaca* and *E. coli* and tested the ability of cell free culture supernatant from the Wabash *Pseudomonas* field isolate to limit growth of these strains at their optimal growth temperatures (30 °C for *Sarcina* and 37 °C for *E. coli). P. fluorescens* growth was selected as a negative control and tested under the same conditions. Over a 54 hour period, the Wabash *Pseudomonas* filtrate completely inhibited growth of *S. aurantiaca* (OD_{600} at hour 54 [Wabash *Pseudomonas:* 0.023 SD = 0.013, unfiltered LB: 1.999 SD = 0.000]) and caused a substantial decrease in *E. coli* growth rate especially during the first 9 hours (OD_{600} at hour 9 [Wabash *Pseudomonas:* 0.023 SD = 0.013, unfiltered LB: 1.999 SD = 0.000]) (Fig. 4). By comparison, the impacts on *P. fluorescens* were minimal as expected: *P. fluorescens* grown in the Wabash *Pseudomonas* filtrate showed a slightly slower growth rate than when grown in LB for the first 21 hours (OD_{600} at hour 21 [Wabash *Pseudomonas:* 1.500 SD = 0.053,

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unfiltered LB: 1.999 SD = 0.000]) , but the two conditions ultimately grew to equivalent titers by 27 hours post inoculation (OD_{600} at hour 27 [Wabash *Pseudomonas:* 1.999 SD = 0.000, unfiltered LB: 1.999 SD = 0.000]).

The Wabash Pseudomonas's antimicrobial profile is distinct from that of P. fluorescens and includes a color impact on Kocuria

Unexpectedly, the Wabash *Pseudomonas* isolate prevented the development of yellow pigmentation in

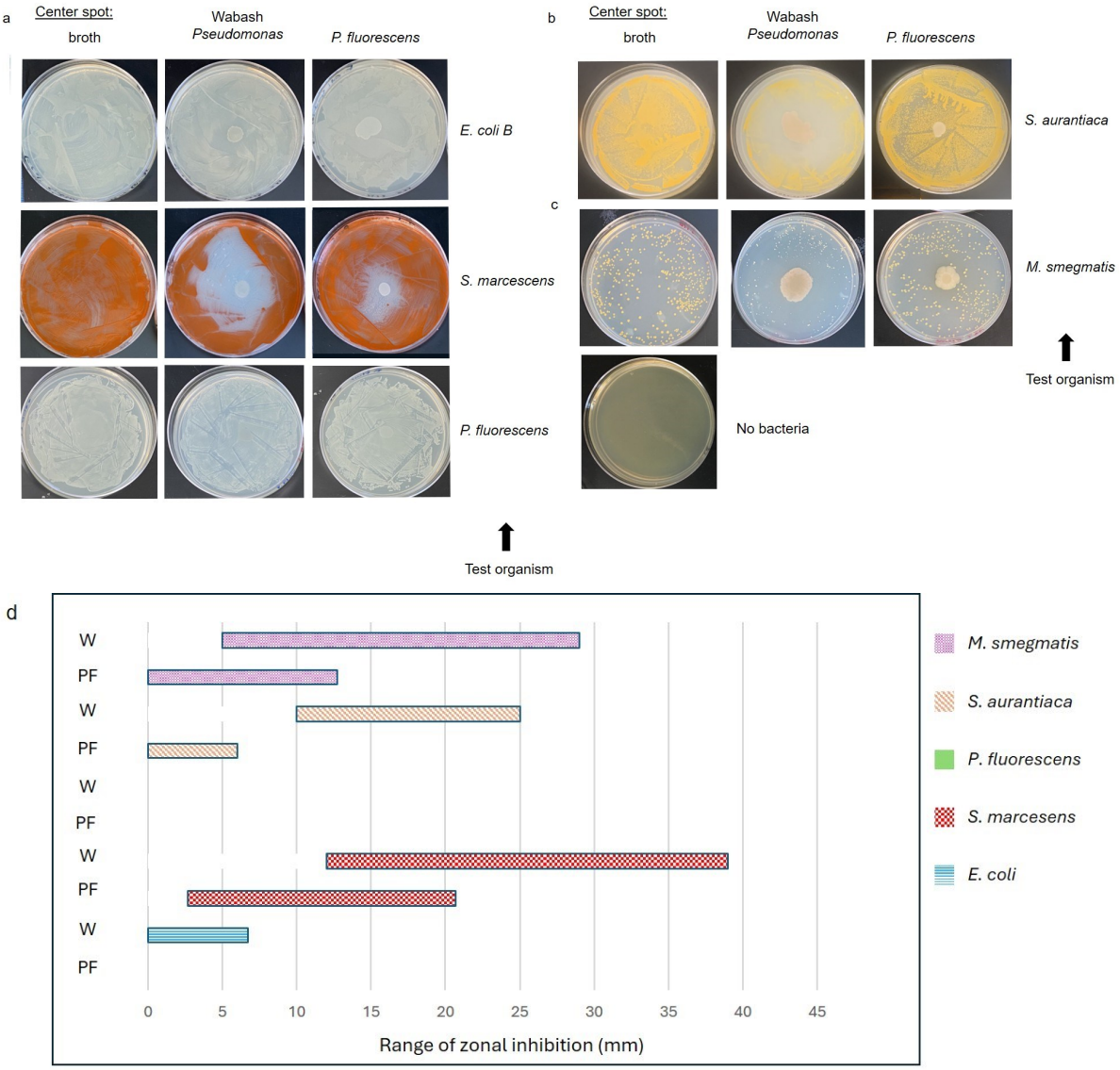

Fig. 3 Range of inhibition by Wabash *Pseudomonas* and *P. fluorescens* on test organisms. Test organisms were spread onto agar plates prior to spotting in the center with broth, Wabash *Pseudomonas*, or *P. fluorescens*. The test organisms are indicated to the right of each row. The organisms spotted on the center of each plate are indicated at the top of each corresponding column. (a) Gram negative organisms and sterility control after 7 days on nutrient agar. (b) Gram positive *S. aurantiaca* after 7 days on nutrient agar (c) Acid fast *Mycobacterium smegmatis* after 8 days on LB agar. Each plate experiment was run in triplicate and representative results are shown. (d) The average zonal inhibition range was calculated for each experiment and each range is shown as a bar on the graph with standard deviations shown on the bars. Test organisms are indicated by color in the figure key. The range of inhibition was calculated by measuring the shortest and longest distance between the edge of the center spotted organism's growth to the edge of the test bacteria. These measurements were taken on each plate and the three low end measurements were averaged and the three high end measurements were averaged to determine the average range of zonal inhibition. "PF" indicates plates spotted with *P. fluorescens* and "W" indicates plates spotted with Wabash *Pseudomonas*. If no bar is present then no inhibition was observed.

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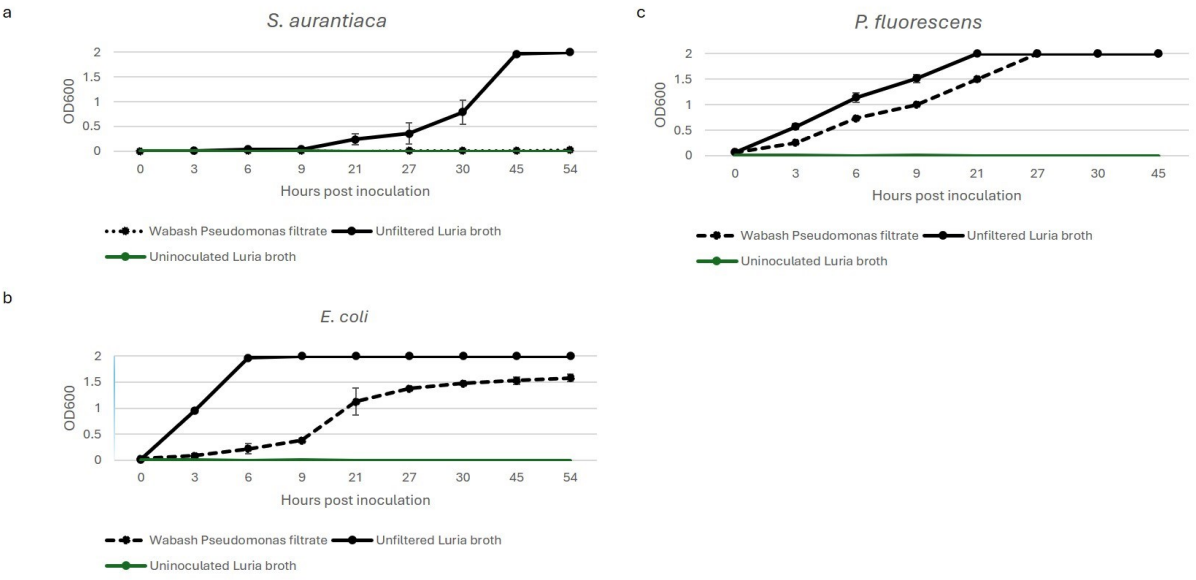

Fig. 4 Inhibition of *S. aurantiaca* and *E. coli* as assessed by cell free culture fluid growth curve assays. Triplicate samples of 0.45um Wabash *Pseudomonas* filtrate or unfiltered LB were inoculated with (a) *S. aurantiaca* (room temperature), (b) *E. coli* (37°C), or (c) *P. fluorescens* (room temperature) prior to assessing OD_{600} at the time indicated on the x axis. Uninoculated LB was run as a sterility control. Error bars show standard deviation of the triplicate samples at each designated time point.

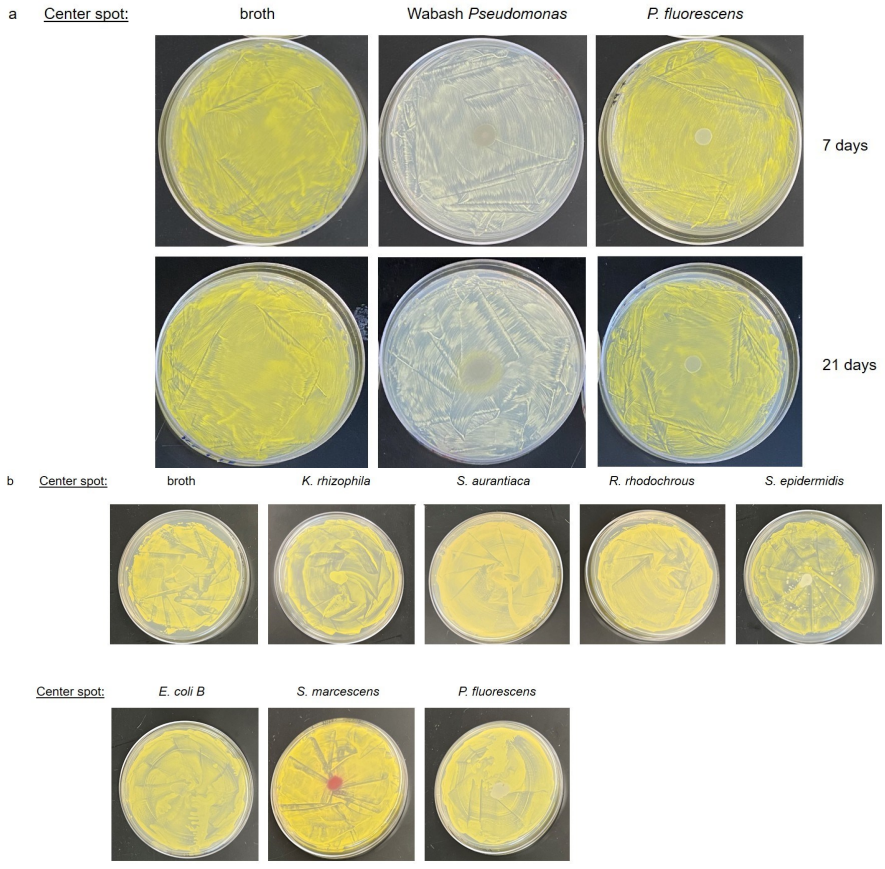

Fig. 5 Impact of Wabash *Pseudomonas* and other Gram positive and Gram negative bacteria on *K. rhizophila* color. Plates were spread with *K. rhizophila* and immediately spotted in the center (a) with broth, the Wabash field isolate, or *P. fluorescens* and photographed on days 7 and 21 or (b) with the organism indicated at the top of each image, photographed on day 7.

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the *K. rhizophila*—a change that was not present on *K. rhizophila* treated with *P. fluorescens* (Fig. 5a). The cream-colored (i.e. not golden) *Kocuria* phenotype emerged on Wabash *Pseudomonas*-spotted plates as soon as *K. rhizophila* growth was detectable and was retained completely through 7 days of room temperature incubation. By 21 days after inoculation, a small ring of yellow emerged immediately next to the region where our *Pseudomonas* isolate had been spotted onto the *K. rhizophila* plate on day 1, but the rest of the *K. rhizophila* growing on the plate remained cream colored (Fig. 5a). In comparison, *K. rhizophila* remained a bright golden yellow throughout the 21 day experiment on plates spotted with broth or *P. fluorescens* (Fig. 5a). The inhibition of *K. rhizophila* pigmentation was not observed on *K. rhizophila* lawns spotted in the center with a variety of other Gram negative or Gram positive organisms (Fig. 5b).

Discussion

In this article we demonstrate a *Pseudomonas* field isolate's inhibition of *E. coli, K. rhizophila, M. smegmatis, S. aurantiaca, S. marcescens,* and *S. epidermidis*. Two of these organisms—*Serratia* and *Kocuria*--are from genera individually known to enhance plant growth or to be involved in soil remediation [6, 20, 21]. Our results thus highlight the complexities of *Pseudomonas*-based biofertilizer optimization: Given the vast diversity of rhizobial microbes, shifting the localized balance of organisms may yield desirable outcomes but also unintended consequences. For instance, might *Pseudomonas*-based biofertilizers [8, 22-23] inhibit indigenous *Serratia* or *Kocuria* PGPBs in soil? If so, what would be the short- and long-term impacts on crop growth?

Interestingly, several of the targets of the Wabash *Pseudomonas* from our experiments are themselves members of genera with robust antimicrobial properties, sometimes even against *Pseudomonas* strains. For example, *Kocuria's* yellow carotinoid pigment functions as an antifungal as well as inhibiting *Staphylococcus aureus, Bacillus subtilis, E. coli,* and *Pseudomonas aeruginosa* (*P. aeruginosa*) [20]. Similarly, the red prodigiosin pigment produced by *S. marcescens* inhibits a wide array of Gram negative and Gram positive bacteria including *Klebsiella pneumoniae*, *Aeromonas hydrophila*, *E. coli*, *Proteus mirabilis*, *Salmonella typhimurium*, *Proteus vulgaris*, *Salmonella enteritidis*, *Corynebacterium glutamicum*, *P. aeruginosa*, *Enterococcus faecalis*, *Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus [11]*. Combined, our data and the literature suggest that inter-relationships of these organisms in soil or in human infections likely change depending on the relative local concentrations of each bacterium and the extent to which each bacterium's antimicrobial genes are expressed. This fluidity is particularly expected for strains such as *Serratia* and *Pseudomonas,* whose virulence factor production is regulated by comparable Acyl-homoserine lactone quorum sensing molecules (secreted molecules used to communicate cell density between cells) and whose antimicrobial (and by extension, perhaps plant growth promoting) features therefore can be altered by structurally related environmental factors [24-25].

The hypothesis of context-dependent interactions between *Pseudomonas, Serratia,* and *Kocuria* strains is consistent with the concentration-dependent effect of the Wabash *Pseudomonas* isolate. For example in the cross-streak assay when our field isolate was given a 48h head start growing on the agar plate before adding the test organisms, the Wabash *Pseudomonas* strain completely inhibited growth of *S. aurantiaca, K. rhizophila,* and *E. coli B* as assessed 5 days after adding the test organisms (Fig. 1). When co-inoculated at the same time in the zonal inhibition assays, however, the Wabash *Pseudomonas* strain yielded clear zones of inhibition against *S. aurantiaca* (10.0 mm SD = 6.1 mm to 15.0 mm SD = 7.0 mm), and *E. coli B* (0.0 mm SD = 0.0 mm to 6.7 mm SD = 5.9 mm), but changed the color of *Kocuria* growth rather than preventing growth of this species (Figs. 3 and 5). Similarly, the impact of our *Pseudomonas* isolate on *Serratia* was nuanced. In the region of the cross-streak plate closest to the Wabash *Pseudomonas's* horizontal streak—and therefore in the area of presumed highest concentration of a diffusible *Pseudomonas*-secreted antimicrobial—*S. marcescens* grew with lower density and less saturated color than on the control plate (Fig. 1). In comparison, in the outermost region of the plate where the *Pseudomonas* antimicrobial concentration would be expected to be lower, the inhibition of *Serratia* lessened as exhibited by the width of the *Serratia* vertical streak as well as its bright red color. In contrast to the cross-streak assay, we never observed white *Serratia* growth in the zonal inhibition assay (Fig. 3). There, our Wabash *Pseudomonas* isolate (12.0 mm SD = 1.7 mm to 27.0 mm SD = 8.2 mm) and *P. fluorescens* (2.7 mm SD = 3.8 mm to 18.0 mm SD = 12.5 mm) both either fully inhibited *Serratia* growth (yielding clear zones of inhibition) or did not inhibit, depending on the diffusion radius from the *Pseudomonas* central spot; in these conditions there was no intermediate area of low growth of white *Serratia* (Fig. 3). Hence, the Wabash *Pseudomonas* isolate's impact varied depending on proximity to the other species and timing of the target addition.

The mechanism and consequences of the Wabash *Pseudomonas*-induced color change in *K. rhizophila*

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remain to be determined. Since pH can alter bacterial growth and metabolism [26], Wabash *Pseudomonas* may cause a change in pH inducing a color change in *K. rhizophila* [27]. Given that *K. rhizophila* coloration typically stems from carotenoid pigments [20, 28], another reasonable but as yet untested hypothesis is that the Wabash *Pseudomonas* strain alters a carotenoid synthesis pathway. If so, it is possible that the color change is a byproduct of a metabolic shift or—since *K. rhizophila's* pigments sometimes function as antimicrobials [20]--perhaps *Pseudomonas* is defending itself by turning off pigment synthesis in its target. Additional experiments are needed to elucidate these options.

Although this manuscript primarily focuses on the rhizomicrobiome applications of our data, we would be remiss to omit potential clinical benefits. The battle against the World Health Organization's (WHO's) multi- and extensively-resistant Priority Pathogens will require a diversity of approaches: ligand and structural target-based design, high efficiency chemical and genetic screens, optimization of multi-drug regimens, and—perhaps surprisingly—old fashioned soil screens. Although the latter are best known from the era of harvesting actinomycetes [29], nature-based drug discovery continues to yield fruit as new locations are sampled and new methods are developed to identify antimicrobials [30-31]. Continuing this line of research, our studies of the Wabash *Pseudomonas* soil isolate confirm previously published reports of *Pseudomonas* inhibition of *Mycobacterium* and *Staphylococcus* [12, 32-37], common models for the WHO priority pathogens *Mycobacterium tuberculosis* and methicillin resistant *Staphylococcus aureus* (Figs. 1-3). To our knowledge this paper also is the first to demonstrate *Pseudomonas* production of antimicrobials that are capable of inhibiting or changing the color of *K. rhizophila* depending on conditions—suggesting that even though high tech drug design is essential in the fight against antibiotic resistance, environmental screens may continue to reveal new secrets (Figs. 1 and 5). In addition, although *P. aeruginosa's* pyoverdine pigment has been demonstrated to inhibit *Sarcina luteus* [38-39], to our knowledge the Wabash isolate is the first *Pseudomonas* with documented inhibition of *S. aurantiaca*. *Sarcina, Serratia,* and *Kocuria* each can cause rare but problematic opportunistic human infection [40-42]. Additional treatments for such infections necessitate an increasingly deep antibiotic pool as drug resistance emerges. Based on our data, follow-up experiments should test existing *Pseudomonas*-derived approved therapeutics for efficacy against these organisms.

Conclusion

In this article we describe the antimicrobial profile of a *Pseudomonas* field isolate obtained on the Wabash College campus in Crawfordsville, Indiana. This strain inhibits growth and/or changes the growth morphology of two other PGPBs whose inhibition by *Pseudomonas* has not been published: *S. marcescens* and *K. rhizophila*. The work emphasizes the continued value of soil screening for investigating organisms that could be used in synthetically produced biofertilizer consortia as well as for identifying potentially novel therapeutics to treat *Serratia, Sarcina,* or *Kocuria* disease in humans.

Authors' Contributions

The authors all contributed in designing and carrying out the experiments described in this manuscript. At the time of the research, Henry G Giesel, Troy T Brown, Samer A Halabi, and Benjamin A Jansen were undergraduates working in Dr. Anne G Bost's research laboratory at Wabash College.

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References

1. Wang T, Xu J, Chen J, Liu P, Hou X, Yang L, Zhang L. (2024) Progress in Microbial Fertilizer Regulation of Crop Growth and Soil Remediation Research. Plants 13(3):346. https://doi.org/10.3390/plants13030346

2. Daniel AI, Fadaka AO, Gokul A, Bakare OO, Aina O, Fisher S, Burt AF, Mavumengwana V, Keyster M, Klein A (2022) Biofertilizer: The future of food security and food safety. Microorganisms 10(6): 1220. https://doi.org/10.3390/microorganisms10061220

3. Dimkić I, Janakiev T, Petrović M, Degrassi G, Fira D (2022) Plant-associated Bacillus and Pseudomonas antimicrobial activities in plant disease suppression via biological control mechanisms - A review. Physiological and Molecular Plant Pathology 117:101754. https://doi.org/10.1016/j.pmpp.2021.101754

4. Bianco C (2024) Plant-Growth-Promoting Bacteria. *Plants* 13(10):1323. https://doi.org/10.3390/plants13101323

5. Igiehon BC, Babalola OO, Hassen AI (2024) Rhizosphere competence and applications of plant growth-promoting rhizobacteria in food production – A review. Scientific African 23:e02081. https://doi.org/10.1016/j.sciaf.2024.e02081

6. Kulkova I, Wróbel B, J Dobrzyński (2024) Serratia spp. as plant growth-promoting bacteria alleviating salinity, drought, and nutrient imbalance stresses. Front. Microbiol. Sec. Microbe and Virus Interactions with Plants 15. https://doi.org/10.3389/fmicb.2024.1342331

7. Singh A, Yadav VK, Chundawat RS, Soltane R, Awwad NS, Ibrahium HA, Yadav KK, Vicas SI (2023) Enhancing plant growth promoting rhizobacterial activities through consortium exposure: A review. Front. Bioeng. Biotechnol 11:1099999. https://doi.org/10.3389/fbioe.2023.1099999

8. Mehmood N, Mahnoor S, Zafarullah S, Hyder S, Rizvi ZF, Gondal AS, Jamil N, Iqbal R, Ali B, Ercisli S, Kupe M (2023) Multifaceted Impacts of Plant-Beneficial *Pseudomonas* spp. in Managing Various Plant Diseases and Crop Yield Improvement. ACS Omega 8(25): 22296–22315. https://doi.org/10.1021/acsomega.3c00870

9. North America Biofertilizer Market Report by Type, Crop, Microorganism, Mode of Application, and Country 2024-2032 (2024) https://www.researchandmarkets.com/report/north-america-biofertilizer-market. Accessed 18 June 2024

10. Hasegawa T, Wakatsuki H, Ju H, Vyas S, Nelson GC, Farrell A, Deryng D, Meza F, Makowski D (2022) A global dataset for the projected impacts of climate change on four major crops. Sci Data 9:58. https://doi.org/10.1038/s41597-022-01150-7

11. Arivuselvam R, Dera A, Ali SP, Alraey Y, Saif A, Hani U, Ramakrishnan SA, Azeeze MTSA, Rajeshkuman R, Susil A, Harindranath H, Kumar BRP (2023) Isolation, Identification, and Antibacterial Properties of Prodigiosin, a Bioactive Product Produced by a New *Serratia marcescens* JSSCPM1 Strain: Exploring the Biosynthetic Gene Clusters of *Serratia* Species for Biological Applications. Antibiotics (Basil) 12(9): 1466. https://doi.org/ 10.3390/antibiotics12091466

12. Serafim B, Bernardino AR, Freitas F, Torres CAV (2023) Recent Developments in the Biological Activities, Bioproduction, and Applications of Pseudomonas spp. Phenazines. Molecules 28(3): 1368. https://doi.org/10.3390/molecules28031368

13. Sousa CA, Ribeiro M, Vale F, Simões M (2024) Phenazines: Natural products for microbial growth control. hLife 2(3): 100-112. https://doi.org/10.1016/j.hlife.2023.11.005

14. Srisangchun, K, Surachat K, Nantavisai K, Pringsulaka O, Sarawaneeyaruk S (2023) Genomic insights into antimicrobial-producing Pseudomonas aeruginosa SWUC02. 13th International Conference on Bioscience, Biochemistry and Bioinformatics (ICBBB) pp.17-22. https://doi.org/10.1145/3586139.3586142

15. Timofeeva AM, Galyamova MR, Sedykh SE (2023) Plant Growth-Promoting Bacteria of Soil: Designing of Consortia Beneficial for Crop Production. Microorganisms 11(12): 2864. https://doi.org/10.3390/microorganisms11122864

16. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) "Basic local alignment search tool." J. Mol. Biol.

doi: 10.29173/spectrum294

215:403-410. https://doi.org/10.1016/S0022-2836(05)80360-2

17. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25(17):3389-402. https://doi.org/10.1093/nar/25.17.3389

18. Kwon SW, Kim JS, Park IC, Yoon SH, Park DH, Lim CK, Go, SJ (2003) Pseudomonas koreensis sp. nov., Pseudomonas umsongensis sp. nov. and Pseudomonas jinjuensis sp. nov., novel species from farm soils in Korea. Int. J. Syst. Evol. Microbiol. 53(1):21-27. https://doi.org/10.1099/ijs.0.02326-0

19. Carvajal, F (1947). Screening Tests for Antibiotics. Mycologia, 39(1), 128–130. https://doi.org/10.2307/3755295

20. Ibrahim GS, El-Shall FN, Arafa AA, Shalabi A, El Awady, M (2024). Bio-production and characterization of carotenoid yellow pigment from Kocuria sp. GMA and exploring its sustainable antioxidant, antimicrobial and antibiofilm properties. *Egyptian Journal of Chemistry 67*(8): 57-68. https://doi.org/10.21608/ejchem.2024.279957.9526

21. Mauceri A, Puccio G, Faddetta T, Abbate L, Polito G, Caldiero C, Renzone G, Lo Pinto M, Alibrandi P, Vaccaro E, Abenavoli MR, Scaloni A, Sunseri F, Cavalieri V, Piccionello AP, Gallo G, Mercati F (2024) Integrated omics approach reveals the molecular pathways activated in tomato by Kocuria rhizophila, a soil plant growth-promoting bacterium. Plant Physiol Biochem 210:108609. https://doi.org/10.1016/j.plaphy.2024.108609

22. Ali MA, Ahmed T, Ibrahim E, Rizwan M, Chong KP, Yong JWH (2024) A review on mechanisms and prospects of endophytic bacteria in biocontrol of plant pathogenic fungi and their plant growth-promoting activities. Heliyon 10 (11). https://doi.org/10.1016/j.heliyon.2024.e31573

23. Zhou L, Höfte M, Hennessy RC (2024) Does regulation hold the key to optimizing lipopeptide production in *Pseudomonas* for biotechnology? Front Bioeng Biotechnol.12:1363183. https://doi.org/10.3389/fbioe.2024.1363183

24. Gonzales M, Jacquet P, Gaucher F, Chabrière E, Plener L, Daudé D (2024) AHL-Based Quorum Sensing Regulates the Biosynthesis of a Variety of Bioactive Molecules in Bacteria. J. Nat. Prod. 87(4): 1268–1284. https://doi-org.wcproxy.palni.edu/10.1021/acs.jnatprod.3c00672

25. Liu Y, J-J Li, H-Y Li, S-M Deng, A-Q Jia (2021) Quorum sensing inhibition of hordenine analogs on *Pseudomonas aeruginosa* and *Serratia marcescens*. Synth Syst Biotechnol. 6(4):360–368. https://doi.org/10.1016/j.synbio.2021.09.01073

26. Ratzke C, Gore J (2018) Modifying and reacting to the environmental pH can drive bacterial interactions. PLoS Biology, 16(3). https://doi.org/10.1371/journal.pbio.2004248

27. Scales BS, Dickson RP, LiPuma JJ, Huffnagle GB (2014) Microbiology, Genomics, and Clinical Significance of the *Pseudomonas fluorescens* Species Complex, an Unappreciated Colonizer of Humans. Clin Microbiol Rev. 27(4): 927–948. https://doi: 10.1128/CMR.00044-14

28. Metwally RA, El-Sersy NA, El Sikaily A, Sabry SA, Ghozlan HA (2022) Optimization and multiple in vitro activity potentials of carotenoids from marine Kocuria sp. RAM1. Sci Rep 12:18203. https://doi.org/10.1038/s41598-022-22897-4

29. Lewis K (2020) The Science of Antibiotic Discovery. Perspective 181(1): 29-45. https://doi.org/10.1016/j.cell.2020.02.056

30. MacNair CR, Tsai CN, Rutherford ST, Tan MW (2023) Returning to Nature for the Next Generation of Antimicrobial Therapeutics. Antibiotics (Basel) 12(8):1267. https://doi.org/10.3390/antibiotics12081267

31. Martínez-Fructuoso L, Arends SJR, Freire VF, Evans JR, DeVries S, Peyser BD, Akee RK, Thornburg CC, Kumar R, Ensel S, Morgan GM, McConachie GD, Veeder N, Duncan LR, Grkovic T, O'Keefe BR (2023) Screen for New Antimicrobial Natural Products from the NCI Program for Natural Product Discovery Prefractionated Extract Library. ACS Infect Dis. 9(6):1245-1256. https://doi.org/10.1021/acsinfecdis.3c00067

32. Machan ZA, Pitt TL, White W, Watson D, Taylor GW, Cole PJ, Wilson R (1991) Interaction between Pseudomonas aeruginosa and Staphylococcus aureus: description of an antistaphylococcal substance. J of Medical Microbiology 34(4). https://doi.org/10.1099/00222615-34-4-213

33. Buber E, Stindl A, Acan NL, Kocagoz T, Zocher R (2002) Antimycobacterial activity of lipodepsipeptides

doi: 10.29173/spectrum294

produced by Pseudomonas syringae. pv syringae B359. Natural Product Letters 16(6): 419-423. https://doi.org/10.1080/10575630290034294

34. Mukherjee K, Mandal S, Mukhopadhyay B, Mandal NC, Sil AK (2014) Bioactive compound from Pseudomonas synxantha inhibits the growth of Mycobacteria. Microbiological Research169(9–10):794-802. https://doi.org/10.1016/j.micres.2013.12.005

35. Hotterbeekx A, Kumar-Singh S, Goossens H, Malhotra-Kumar S (2017) In vivo and In vitro Interactions between Pseudomonas aeruginosa and Staphylococcus spp. Front. Cell. Infect. Microbiol. Sec. Molecular Bacterial Pathogenesis 7. https://doi.org/10.3389/fcimb.2017.00106

36. Biswas L, Götz F (2022) Molecular Mechanisms of Staphylococcus and Pseudomonas Interactions in Cystic Fibrosis. Front. Cell. Infect. Microbiol, Sec. Molecular Bacterial Pathogenesis 11. https://doi.org/10.3389/fcimb.2021.824042

37. Kaur M, Jangra M, Singh H, Tambat R, Singh N, Jachak SM, Mishra S, Sharma C, Nandanwar H and Pinnaka AK (2019) Pseudomonas koreensis Recovered From Raw Yak Milk Synthesizes a Carboline Derivative With Antimicrobial Properties. Front. Microbiol. 10:1728. https://doi.org/10.3389/fmicb.2019.01728

38. Poiata A, Vlahovici A, Creanga DE (2005) Ferrofluid effect on Pseudomonas pyoverdine. Journal of Magnetism and Magnetic Materials 289(12): 455-458. https://doi.org/10.1016/j.jmmm.2004.11.128

39. Poiata A, Creanga D (2015). Magnetic Nanoparticle Influence on Pseudomonas Metabolites with Antimicrobial Properties. Romanian Journal of Physics 60:228-236

40. Tartaglia D, Coccolini F, Mazzoni A, Strambi S, Cicuttin E, Cremonini C, Taddei G, Puglisi AG, Ugolini C, Di Stefano I, Basolo F, Chiarugi M (2022) *Sarcina Ventriculi* infection: a rare but fearsome event. A Systematic Review of the Literature. International Journal of Infectious Diseases 115:48-61. https://doi.org/10.1016/j.ijid.2021.11.027

41. Ziogou A, Giannakodimos I, Giannakodimos A, Baliou S, Ioannou P (2023) *Kocuria* Species Infections in Humans—A Narrative Review. Microorganisms 11(9): 2362. https://doi.org/10.3390/microorganisms11092362

42. Zivkovic Zaric R, Zaric M, Sekulic M, Zornic N, Nesic J, Rosic V, Vulovic T, Spasic M, Vuleta M, Jovanovic J, Jovanovic D, Jakovljevic S, Canovic P (2023) Antimicrobial Treatment of Serratia marcescens Invasive Infections: Systematic Review. *Antibiotics* 12(2):367. http://doi.org/10.3390/antibiotics12020367

doi: 10.29173/spectrum294