The Biotechnological Potential of Bacteria Isolated From Environmental Samples of Mines and Reserve Areas in Honduras

Alejandra Melgas Valeriano , Mario Rietti-Osorio , Paola Valle-Ordoñez , David Mejía , Tanya Saray Ordoñez Martínez , Andrés S. Ortiz-Morazán *

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Article

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Alejandra Melgar Valeriano [1], Mario Rietti-Osorio [1], Paola Valle-Ordoñez [1], David Mejía [1], Tanya Ordoñez [2] and Andrés S. Ortiz-Morazán [1,3,*]

[1] Escuela de Microbiología, Facultad de Ciencias, Universidad Nacional Autónoma de Honduras
[2] Escuela de Física, Facultad de Ciencias, Universidad Nacional Autónoma de Honduras
[3] Centro de Investigaciones en Agentes Infecciosos y Zoonóticos (CIAIZ), Instituto de Investigaciones en Microbiología, Facultad de Ciencias, Universidad Nacional Autónoma de Honduras
* Correspondence: aortizm@unah.edu.hn

Abstract: Biotechnology provides sustainable solutions for soil and ecological deterioration resulting from human activities. This study investigated the biotechnological potential of strains isolated from environmental samples collected in protected areas and mines of Honduras, focusing on their application for producing biofertilizers and bioremediation. 63 samples were collected from: soil, soil covers, mine waters, and hot springs. From these, 118 bacterial strains were isolated and characterized using microbiological and molecular techniques. The genera *Pseudomonas*, *Bacillus*, *Azospirillum*, and *Azotobacter* were associated with some of the isolated strains and stood out for their ability to solubilize potassium, fix nitrogen and produce bioactive compounds. Isolates related to bacteria of the genera *Bacillus* and *Pseudomonas* demonstrated potential use in biotechnology. Furthermore, some strains exhibited significant genetic diversity and biological similarity to bacterial genera and species utilized in pollution remediation and soil fertility improvement. A small group shows atypical morphologies, including pleomorphic, filamentous structures, features with vacuole-like traits, pigment production, iridescence, and metallic sheen. These results highlight the microbial biodiversity present in soil covers of protected Honduran ecosystems and their importance as reservoirs of microorganisms with biotechnological applications. This study emphasizes the importance of preserving these natural regions, not only for biodiversity preservation but also for utilizing their potential in formulating sustainable solutions to environmental and agricultural challenges.

Keywords: biotechnology; bacteria; biological fertilizers; bioremediation; microbial biodiversity

1. Introduction

According to the FAO, the degradation of soil and associated ecosystems is one of the main challenges facing humanity due to the growing demand for food and therefore for related industries [1]. Human activities have introduced numerous contaminants into the ecosystem, a significant portion of which has contaminated the soil. These contaminants, beyond degrading the ecosystem, may negatively impact the health of plants, animals, and humans [2,3]. Contaminated soil can also contaminate other elements such as water sources and therefore, the health of the communities that benefit from these. It has been demonstrated that primary soil pollutants include industrial activities, mining, solid waste management, agricultural, combustion of fossil fuels, and transportation emissions [4]. Except for agrochemicals, the other pollutants are challenging to quantify, due to technological limitations or their environmental release cycles, which complicate measurement [1]. Ironically, agriculture is essential for the advancement and sustainability of the global population.

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One proposed strategy to mitigate agriculture's impact on ecosystems is the utilization of biological products which may either completely or partially fulfill the nutritional needs of crops and to control or mitigate the impact of pests and disease that affect crops [5]. Microorganisms represent significant biotechnological tools, attributable to their diverse uses across several sectors including agriculture and industry. Bacteria from genera such as *Pseudomonas*, *Bacillus*, and *Azotobacter* have shown utility in sustainable agriculture by promoting plant growth, augmenting soil fertility and by biologically controlling diseases [6]. Moreover, utilizing these microbes as bioremediation agents is an option for reducing soil contamination, utilizing their ability to degrade, transform, or absorb pollutants. Furthermore, the previously described taxa, and the genus *Serratia*, *Proteus*, and *Methylobacterium*, have been evaluated for their bioremediation potential and are usually found in different ecosystems [7,8]. Consequently, the investigation of this and other microorganisms is an important objective for contamination management and the mitigation of ecological deterioration [9]. This study aimed to evaluate several environmental samples to identify optimal matrices for isolating bacteria with biotechnology potential. The project's design primarily employed conventional microbiological techniques, supplemented with molecular tests to enhance bacterial identification from the collected samples.

2. Materials and Methods

Soil, ground cover (leaf litter), hot springs, and mine water samples have been collected from mines, reserved areas and national parks in Honduras (Figure 1). All samples were obtained under aseptic conditions and in accordance with the guidelines described below. Soil samples were obtained about 10 cm deep to evaluate facultative anaerobic bacteria. Soil cover samples were taken 10 cm from the trunks of various plant types. In the end, hot spring and mine water samples were obtained using plastic containers previously sterilized guiding with the Standard Methods for Examination of Water and Wastewater 23rd Edition [10]. The samples were transported through cold chain to the laboratory of the Center for Research on Infectious and Zoonotic Agents (CIAIZ-UNAH), where they were then diluted in 0.1% buffered peptone water at a 1/10 ratio until obtaining a dilution of 10^{-8}. The samples were pre-enriched by inoculating conical tubes with sterile LB Broth, using 1 ml of the 10^{-6} and 10^{-8} dilutions. The samples were afterwards incubated for 24 to 72 hours at room temperature with constant agitation. LB Agar and Ashby Agar plates (10 grams of mannitol, 0.2 grams of K_2HPO_4, 0.2 grams of $MgSO_4$, 0.2 grams of NaCl, 0.1 grams of $CaSO_4$, 5.0 grams of $CaCO_3$, 2% of agar; pH 7.5 ±0.2 for 1 liter) [11] were inoculated with 0.1 ml of pre-enriched cultures by spreading on the surface and incubated at 25°C for 24 hours or until bacterial colonies emerged. Subsequently, bacteria isolated from the previous phase were selectively cultured using the Frobisher technique on LB agar at periodic times until pure strains were obtained. The isolates have been studied morphologically, biochemically, and genetically to identify the main genus and species that were isolated. Morphological characterization was performed by staining methods and the descriptions of the identified colonies. Biochemical characterization was done with biochemical assays depending on the presumptive bacterial species. The tests conducted included biological nitrogen fixation in Ashby agar, potassium solubilization in Aleksandrow agar (0.1 grams of bromothymol blue, 5.0 grams of dextrose, 0.005 grams of $FeCl_3$, 0.1 grams de $CaCO_3$, 0.5 grams of $MgSO_4 * 7H_2O$, 2.0 grams of $CaSO_4$, 2.0 grams of K-bearing minerals, 2.0 grams of K_2HPO_4 and 2% of agar; for 1 liter) [12], carbohydrate fermentation (which includes xylose, mannitol, saccharose, maltose, glucose, lactose, and arabinose), indole test, ornithine decarboxylation, motility, growth in different NaCl concentrations, utilization of citrate as a carbon source, nitrite reduction, and assay of catalase activity. Lastly, to identify some species and genera of relevance like *Bacillus*, *Pseudomonas*, genetic markers associated with speciation were amplified, DNA samples were extracted and purified using the CTAB method [13]. Different regions of gene 16S rRNA were subsequently amplified by PCR as detailed below. To confirm the *Pseudomonas* genus, specific primers F311Ps (5'CTGGTCTGAGAGGATGATCAGT3') and R1459Ps (5'AATCACTCCGTGGTAACCGT3') were

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employed [14]. Amplification was conducted in an Applied Biosystems thermal cycler with the subsequent protocol: One cycle at 94°C for 5 minutes. 35 cycles at 94°C for one minute, 62°C for one minute, and 72°C for one minute, followed by a final extension at 72°C for ten minutes. For confirmation of the *Bacillus* genus, primers BK1F (5'TCACCAAGGCAACGATGCG'3) and BK2F (5'CGTATTCACCGCGGCATG'3) were employed [15]. PCR was conducted using an Applied Biosystems thermocycler with the subsequent protocol: One cycle at 94°C for 5 minutes, followed by 30 cycles of 94°C for 1 minute, 56°C for One minute, and 72°C for one minute, followed by a final extension at 72°C for ten minutes. A final volume of 20 µl was established for all PCR reaction mixes, which included 1 µl of genomic DNA, 10 µl of Apex Master Mix (Cat: 42-134B. Tris-HCl pH 8.5, $(NH_4)_2SO_4$, 0.2% Tween 20, 0.4 mM of each dNTP, Apex Taq DNA Polymerase), 0.2 of each primer 10µM, and MilliQ water. Finaly, PCR products were evaluated on 2% agarose gels.

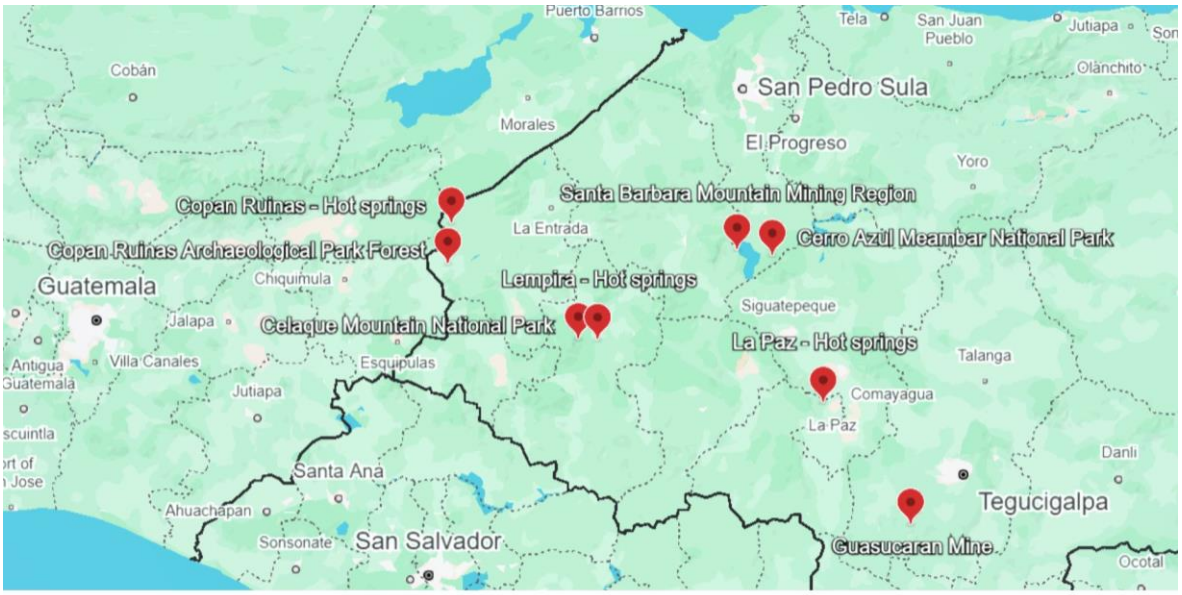

Figure 1: Locations for the collection of environmental samples utilized to isolate bacteria with biotechnological potential.

3. Result

63 samples were obtained, including 22 soil samples, 24 ground cover samples, 15 hot springs and 2 mine water samples. 118 different bacterial strains were isolated, with 75 from LB agar and 43 from Ashby agar. The matrix from which the greatest number of bacterial isolates were obtained was ground cover samples (54 isolates), followed by soil samples (32 isolates), hot spring water (28 isolates) and mine water samples (4 isolates). Regarding cell morphology, most bacteria showed type Gram-negative bacillus cells, with 60 isolates, followed by Gram-positive bacillus, Gram-negative coccus, and Gram-positive coccus which 28, 20, and 10 isolates, respectively. Regarding colonial morphology, the predominant bacterial colonies were color white, rounded edges, circular shapes, clear shiny and pasty surface with 77 isolates, followed by thirteen isolated with similar characteristics to those previously isolates, but with flat surface. Thirteen translucency strains corresponded to those previously observed, while others differed only in the production of pink pigments and the generation of a green metallic sheen. Eleven isolates showed yellow color some strains show a greenish yellow, punctate shape, clear shiny and slightly convex elevation; and one colony exhibited a dry, friable kind. While another showed pasty, opaque colonies of a yellowish-white color. Finaly, three isolations exhibited atypical cellular morphologies, including the strain C.T 19.3 that exhibiting pleomorphic morphology, strain S 11.1 which showed a filamentous morphology and A.T 1.1 which exhibited vacuolar-like features (Figure 2). The biochemical test results (Table 1) indicated findings related to *Bacillus, Pseudomonas, Azsopirillum, Azotobacter,* including other organisms capable of solubilize potassium and fix nitrogen, that is proliferated on highly selective

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media such Ashby and Aleksandrow agar (Figure 3). Furthermore, organisms presumptive to be related to the *Bacillus* genus conducted catalase testing and were grown in media supplemented with 7% NaCl; all results were positive. Moreover, molecular analysis suggested that fourteen strains are classified within the genus *Bacillus* and four within the genus *Pseudomonas* (Figure 4). Finally, other characteristics including pigment production, iridescence, and metallic sheens were noted in nine different strains (Figure 2).

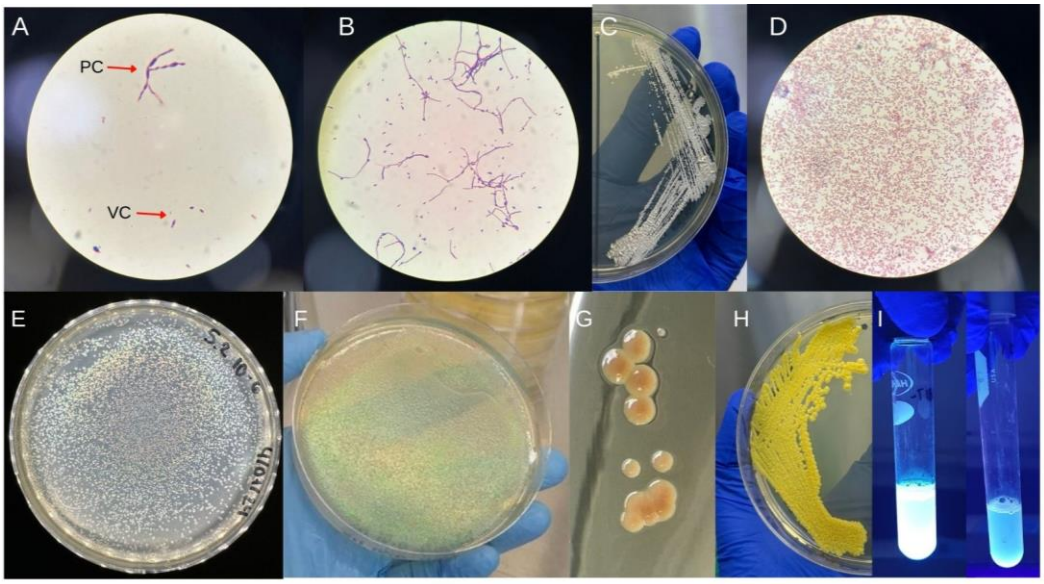

Figure 2: A. Gram stain of strain C.T 19.3, P.C.= pleomorphic cells, V.C.= vegetative cells. **B.** and **C.** Gram stain and colonial morphology of strain S 11.1, respectively. **D.** Strain A.T 1.1 with vacuolar-like features. **E.** Iridescent colonies of strain C.T. 13.1. **F.** Colonies of the C.T. 19.1 strain with metallic shine. **G.** Colonies of C.T. 23.1 strain producing pink pigment, **H.** Colonies of C.T. 15.1 strain producing yellow pigment. **I.** Left: Fluorescent culture of strain CT.18 exposed to UV light, right: negative control.

Table 1. Results of presumptive biochemical tests for the identification of potential bacterial genera associated with isolates from environmental samples.

Sample ID	Carbohydrate Fermentation							Motility	Indole test	Ornithine decarboxylation	Growth in NaCl 6.5%	Citrate Usage	NO3 Reduction
	Xylose	Mannitol	Saccharose	Maltose	Glucose	Lactose	Arabinose						
CT.11.1	-	+	-	+	+	-	-	+	+	+	+	+	+
S.5.3	+	+	+	+	+	-	+	+	+	-	+	-	+
S.26.2	+	-	-	+	+	-	+	+	+	-	-	+	-
AT.5.3	-	+	+	+	+	-	+	+	+	-	+	+	+
CT.4.2	-	+	+	+	+	-	-	+	+	-	+	+	+
CT.15.3	+	-	+	+	-	-	+	-	-	-	-	+	-
CT.5.1	+	+	+	+	+	-	-	+	+	-	-	+	+
CT.12.1	+	-	+	-	+	-	-	-	-	-	-	+	-
CT.18.1	+	+	-	-	+	-	-	+	-	-	+	+	+
CT.6.1	+	+	+	-	+	-	-	+	-	-	+	+	+
S.7.2	+	-	-	-	-	-	+	-	-	-	-	+	-
CT.6.2	-	+	+	+	+	-	-	+	+	-	+	+	+

Note: (+) = Positive test, (-) = Negative test.

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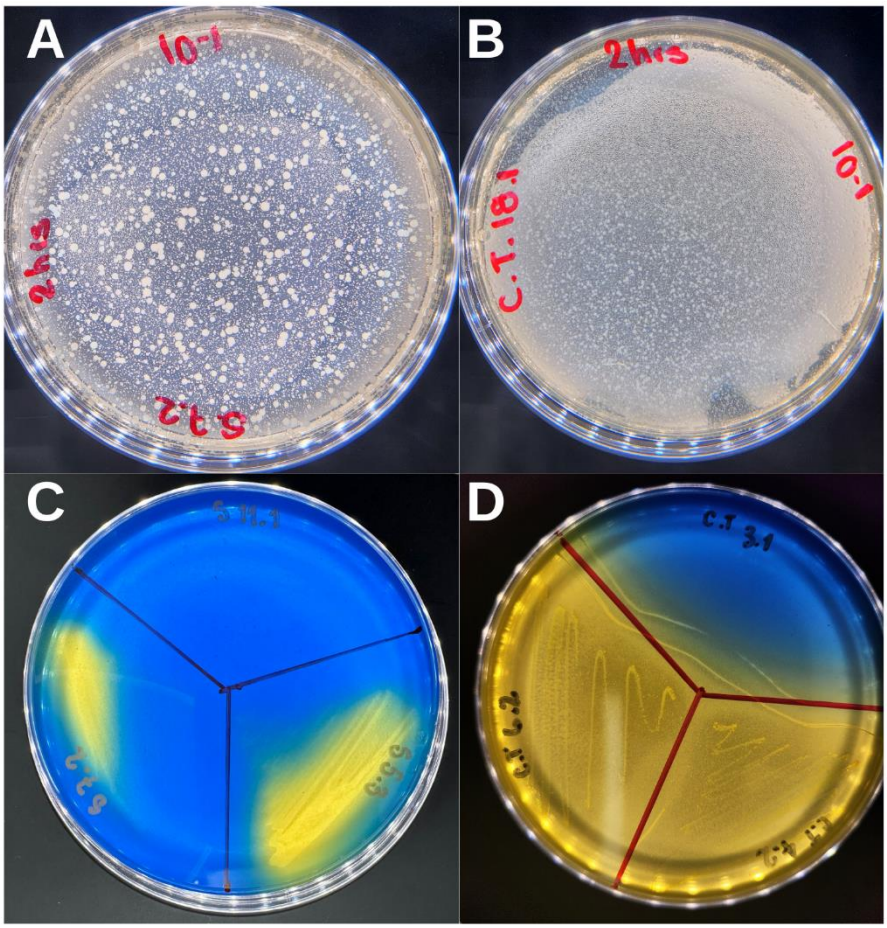

Figure 3: **A** and **B** show different nitrogen-fixing bacterial colonies isolated on Ashby agar. **C** and **D** show different colonies than may generate yellow precipitates on Aleksandrow agar.

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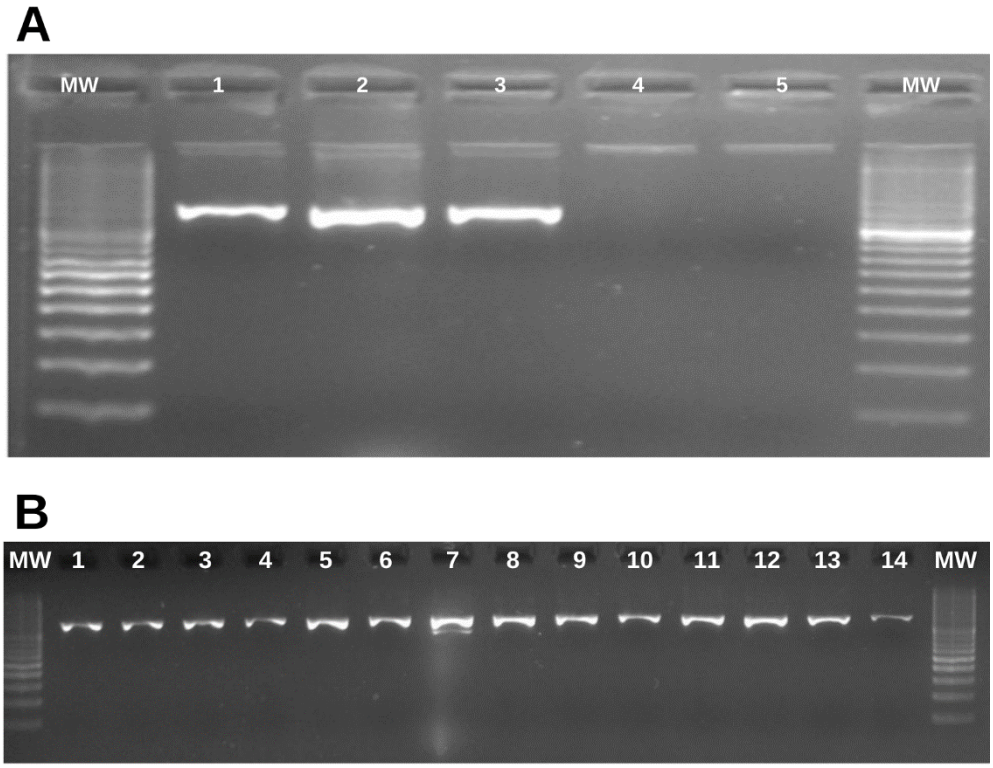

Figure 4: Results from molecular tests for confirming bacterial species with biotechnological potential. **A.** Results for presumed strains of the genus *Pseudomonas*. (**1.** CT.5.1, **2.** CT.6.1, **3.** CT.18.1, **4** and **5** negative controls). **B.** Results for probable strains of the genus Bacillus (**1.** S16.1., **2.** S16.2. **3.**, S18.1., **4.** S18.2. 5. S18.3., **6.** S19.1., **7.** S19.2., **8.** S19.3., **9.** S22.1., **10.** C.T 20.2., **11.** C.T21.2., **12.** C.T 21.3., **13.** C.T24.3. **14.** C.T24.4.

4. Discussion

Our findings indicate that soil cover samples exhibit significant bacterial diversity with biotechnological potential, and they suggest selectivity based on the associated plant species [16]. Different metabolites found in decomposing organic matter may help to explain this [16,17]. Both flora, fauna and associated microbiota may influence the viability of bacterial genera, while simultaneously promoting the proliferation of other bacterial species [18]. Iridescent, metallic shine, and pigment-producing bacteria were obtained from soil cover samples (C.T 13.1, C.T 15.1, C.T 19.1, C.T 23.1) indicating a notable characteristic that may suggest a greater biotechnological potential of the identified bacterial strains. Other bacteria with comparable properties have been isolated from hot spring samples (A.T 2.2, A.T 11.1, A.T 11.2, A.T 14.4, A.M 1). Iridescence and metallic sheens have been described in several research; nevertheless, these characteristics have not been explicitly related to biotechnological applications. Certain genera of bacteria that can provide these features include the genus *Flavobacterium* and *Bacillus* [19,20]. In contrast, the pigment-producing strains may be classified into the genera *Micrococcus* and *Methylobacterium;* multiple species of *Micrococcus* have exhibited the ability to produce compounds with antibacterial, antioxidant, cytogenetic, and cytotoxic properties [21,22]. *Methylobacterium* has been reported in several agricultural applications due to its capacity for biological nitrogen fixation; however, species of this genus have been associated with the degradation of petroleum-derived pollutants in bioremediation tasks [8,23–25]. Conversely, the strain showing vacuole-like structures may be associated with the species *Alkalimicrobium pacificum* [26] and *Polaromonas vacuolata* [27], which have been previously documented for their capacity to produce vesicles or vacuole-like gas structures. In addition, the strain exhibiting cellular pleomorphism may be related to other bacteria that possess similar cellular morphology, including those found in the genera *Hyphobacterium* and *Hyphomicrobium* [28–31]. These genera have been previously described for their bioremediation capabilities, especially in

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denitrification processes [30,32–34]. These findings emphasize the importance of investigating these isolates to elucidate their biotechnological potential. Sixteen strains (CT.4.2, CT.6.2, CT.15.3, S.7.2, S.5.3, AT.5.1, AT.1.2, AT.6.2, AT.8.2, AT.5.1, AT.5.3, S.20.2, S.25.2, S.26.2, S.7.2 y S.5.3) demonstrated the capacity to grow in Aleksandrow agar, producing yellow precipitates that suggest potential for potassium solubilization in agricultural biotechnology. Among these, at least six exhibited significant solubilizing capacity, producing precipitates within the initial 12 to 24 hours following inoculation. Potassium is an important macronutrient for some crops, including citrus and *Musaceae*, where the requirements for potassium and nitrogen are comparable [35,36]. The application of bacteria to supply potassium to plants has demonstrated efficacy in several places worldwide. On the other hand, three strains (CT.5.1, CT.6.1 and CT.18.1) showed the capacity to generate fluorescence in simple medium. This particularity, in addition to its phenotypic and molecular characteristics, suggested potential strains of *P. fluorescens*. This finding is relevant as *P. fluorescens* is associated with the synthesis of phytohormones that can promote plant growth or induce dormancy break among other effects [37–39]. The utilization of *P. fluorescens* as an active component in biofertilizers that promote the growth of plants has been proposed in numerous studies [40–42]. At least fourteen isolates on Ashby agar may show promise in the application of agriculture. The conducted tests suggest bacteria from the genera *Azotobacter* and *Azospirillum*. Bacteria from these genera produce symbiotic interactions with agriculturally significant plants, enhancing nitrogen absorption thus increasing vegetative growth [43–45]. The fourteen identified strains associated with the *Bacillus* genus could have bioremediation potential. The reason for this is that strains of this kind of organism have been used as bioremediation agents in many different environments before. Furthermore, strains of the *Bacillus* genus have demonstrated utility as biocatalysts in several industrial applications [46–48]. Finaly, at least 36% of the identified isolates found had biotechnological potential, indicating that this kind of sample possesses significant microbial diversity for biotechnological exploration. These findings coincide with previous similar research [49,50].

5. Conclusions

The investigation of environmental samples to isolate microorganisms with biotechnological potential is a highly effective method. The sample type and associated flora are essential factors for directing the isolation of the target microorganisms. It is important to expand the investigation of these samples to identify bacteria with potential for biofertilizer and bioremediation agent production, thus contributing to the sustainable development goals concerning food security and environmental sustainability.

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References

1. FAO, UNEP. Summary for policy makers GLOBAL ASSESSMENT OF SOIL POLLUTION [Internet]. Rome, Italy; 2021 [cited 2024 Dec 8]. Available from: https://openknowledge.fao.org/handle/20.500.14283/cb4827en
2. Steffan JJ, Brevik EC, Burgess LC, Cerdà A. The effect of soil on human health: an overview. Eur J Soil Sci. 2018 Jan 17;69(1):159–71.

3. Shetty SS, D D, S H, Sonkusare S, Naik PB, Kumari N S, et al. Environmental pollutants and their effects on human health. Heliyon. 2023 Sep;9(9):e19496.
4. Zhang X, He L, Yang X, Gustave W. Editorial: Soil pollution, risk assessment and remediation. Front Environ Sci. 2023 Jul 18;11.
5. Pirttilä AM, Mohammad Parast Tabas H, Baruah N, Koskimäki JJ. Biofertilizers and Biocontrol Agents for Agriculture: How to Identify and Develop New Potent Microbial Strains and Traits. Microorganisms. 2021 Apr 13;9(4):817.
6. Minuț M, Diaconu M, Roșca M, Cozma P, Bulgariu L, Gavrilescu M. Screening of Azotobacter, Bacillus and Pseudomonas Species as Plant Growth-Promoting Bacteria. Processes. 2022 Dec 28;11(1):80.
7. Olajide PO, Adeloye AO. Hydrocarbon biodegradation by Proteus and Serratia strains isolated from oil-polluted water in Bonny Community, Niger Delta, Nigeria. Results Chem. 2023 Jan;5:100735.
8. Dourado MN, Aparecida Camargo Neves A, Santos DS, Araújo WL. Biotechnological and Agronomic Potential of Endophytic Pink-Pigmented Methylotrophic *Methylobacterium* spp. Biomed Res Int. 2015;2015:1–19.
9. Kebede G, Tafese T, Abda EM, Kamaraj M, Assefa F. Factors Influencing the Bacterial Bioremediation of Hydrocarbon Contaminants in the Soil: Mechanisms and Impacts. J Chem. 2021 Nov 15;2021:1–17.
10. Gilcreas FW. Standard methods for the examination of water and waste water. Am J Public Health Nations Health. 1966 Mar;56(3):387–8.
11. Romero-Perdomo F, Abril J, Camelo M, Moreno-Galván A, Pastrana I, Rojas-Tapias D, et al. Azotobacter chroococcum as a potentially useful bacterial biofertilizer for cotton (Gossypium hirsutum): Effect in reducing N fertilization. Rev Argent Microbiol. 2017 Oct;49(4):377–83.
12. RAJAWAT MVS, SINGH S, TYAGI SP, SAXENA AK. A Modified Plate Assay for Rapid Screening of Potassium-Solubilizing Bacteria. Pedosphere. 2016 Oct;26(5):768–73.
13. Atashpaz S, Khani S, Barzegari A, Barar J, Vahed SZ, Azarbaijani R, et al. A robust universal method for extraction of genomic DNA from bacterial species. Microbiology (N Y). 2010 Aug 20;79(4):538–42.
14. Inceoğlu O, Hoogwout EF, Hill P, van Elsas JD. Effect of DNA Extraction Method on the Apparent Microbial Diversity of Soil. Appl Environ Microbiol. 2010 May 15;76(10):3378–82.
15. Anyi U, New CY, Chai LC, Loo YY, M.A.R. NK, Kayali AY, et al. Prevalence of Bacillus cereus s.l. in ultra-high temperature chocolate milk from selected milk manufacturers in Malaysia. Food Res. 2020 Mar 8;4(4):982–90.
16. Chauhan P, Sharma N, Tapwal A, Kumar A, Verma GS, Meena M, et al. Soil Microbiome: Diversity, Benefits and Interactions with Plants. Sustainability. 2023 Oct 9;15(19):14643.
17. Song Y, Yao S, Li X, Wang T, Jiang X, Bolan N, et al. Soil metabolomics: Deciphering underground metabolic webs in terrestrial ecosystems. Eco-Environment & Health. 2024 Jun;3(2):227–37.
18. Murúa JM, Gaxiola A. Variability in terrestrial litter decomposition can be explained by nutrient allocation strategies among soil decomposer communities. Funct Ecol. 2023 Jun 31;37(6):1642–52.
19. Kientz B, Luke S, Vukusic P, Péteri R, Beaudry C, Renault T, et al. A unique self-organization of bacterial sub-communities creates iridescence in Cellulophaga lytica colony biofilms. Sci Rep. 2016 Jan 28;6(1):19906.
20. Kientz B, Vukusic P, Luke S, Rosenfeld E. Iridescence of a Marine Bacterium and Classification of Prokaryotic Structural Colors. Appl Environ Microbiol. 2012 Apr;78(7):2092–9.
21. Hegazy AA, Abu-Hussien SH, Elsenosy NK, El-Sayed SM, Abo El-Naga MY. Optimization, characterization and biosafety of carotenoids produced from whey using Micrococcus luteus. BMC Biotechnol. 2024 Oct 7;24(1):74.
22. Tizabi D, Hill RT. Micrococcus spp. as a promising source for drug discovery: A review. J Ind Microbiol Biotechnol. 2023 Feb 17;50(1).
23. Zakuan Azizi Shamsul Harumain, Mohd Azrul Naim Mohamad, Noor Faizul Hadry Nordin, Mohd Yunus Abd Shukor. Biodegradation of Petroleum Sludge by Methylobacterium sp. Strain ZASH. Trop Life Sci Res. 2023 Jul 21;34(2).
24. Torres Vera R, Bernabé García AJ, Carmona Álvarez FJ, Martínez Ruiz J, Fernández Martín F. Application and effectiveness of Methylobacterium symbioticum as a biological inoculant in maize and strawberry crops. Folia Microbiol (Praha). 2024 Feb 1;69(1):121–31.

25. Arrobas M, Correia CM, Rodrigues MÂ. Methylobacterium symbioticum Applied as a Foliar Inoculant Was Little Effective in Enhancing Nitrogen Fixation and Lettuce Dry Matter Yield. Sustainability. 2024 May 26;16(11):4512.
26. Zhang G, Yang Y, Wang S, Sun Z, Jiao K. Alkalimicrobium pacificum gen. nov., sp. nov., a marine bacterium in the family Rhodobacteraceae. Int J Syst Evol Microbiol. 2015 Aug 1;65(Pt_8):2453–8.
27. IRGENS RL, GOSINK JJ, STALEY JT. Polaromonas vacuolata gen. nov., sp. nov., a Psychrophilic, Marine, Gas Vacuolate Bacterium from Antarctica. Int J Syst Bacteriol. 1996 Jul 1;46(3):822–6.
28. Ruan CJ, Zheng XW, Wang J, Song L, Zhu YX, Du WB, et al. Hyphobacterium indicum sp. nov., isolated from deep seawater, and emended description of the genus Hyphobacterium. Int J Syst Evol Microbiol. 2018 Dec 1;68(12):3760–5.
29. Zhao S bo, Liu L, Lian F bai, Du ZJ. Hyphobacterium marinum sp. nov. and Hyphobacterium lacteum sp. nov., isolated from marine sediment. Int J Syst Evol Microbiol. 2024 Sep 5;74(9).
30. Martineau C, Villeneuve C, Mauffrey F, Villemur R. Hyphomicrobium nitrativorans sp. nov., isolated from the biofilm of a methanol-fed denitrification system treating seawater at the Montreal Biodome. Int J Syst Evol Microbiol. 2013 Oct 1;63(Pt_10):3777–81.
31. Martineau C, Mauffrey F, Villemur R. Comparative Analysis of Denitrifying Activities of Hyphomicrobium nitrativorans, Hyphomicrobium denitrificans, and Hyphomicrobium zavarzinii. Appl Environ Microbiol. 2015 Aug;81(15):5003–14.
32. Pol A, Op den Camp HJM, Mees SGM, Kersten MASH, van der Drift C. Isolation of a dimethylsulfide-utilizingHyphomicrobium species and its application in biofiltration of polluted air. Biodegradation. 1994 Jun;5(2):105–12.
33. Martineau C, Mauffrey F, Villemur R. Comparative Analysis of Denitrifying Activities of Hyphomicrobium nitrativorans, Hyphomicrobium denitrificans, and Hyphomicrobium zavarzinii. Appl Environ Microbiol. 2015 Aug;81(15):5003–14.
34. Li J, Törkel K, Koch J, Tanabe TS, Hsu HY, Dahl C. In the Alphaproteobacterium Hyphomicrobium denitrificans SoxR Serves a Sulfane Sulfur-Responsive Repressor of Sulfur Oxidation. Antioxidants. 2023 Aug 16;12(8):1620.
35. Thu AM, Alam SM, Khan MA, Han H, Liu DH, Tahir R, et al. Foliar spraying of potassium sulfate during fruit development comprehensively improves the quality of citrus fruits. Sci Hortic. 2024 Dec;338:113696.
36. Simon CA, Kayode PB. Nitrogen and potassium fertilizer influenced nutrient use efficiency and biomass yield of two plantain (Musa spp. AAB) genotypes. Afr J Agric Res. 2015 Feb 5;10(6):458–71.
37. Al-Karablieh N, Al-Shomali I, Al-Elaumi L, Hasan K. Pseudomonas fluorescens NK4 siderophore promotes plant growth and biocontrol in cucumber. J Appl Microbiol. 2022 Sep 1;133(3):1414–21.
38. Wu X, Wang X, Meng H, Zhang J, Lead JR, Hong J. Pseudomonas fluorescens with Nitrogen-Fixing Function Facilitates Nitrogen Recovery in Reclaimed Coal Mining Soils. Microorganisms. 2023 Dec 19;12(1):9.
39. Wang Z, Chen X, Zhong T, Li B, Yang Q, Du M, et al. Bioeffector *Pseudomonas fluorescens* ZX Elicits Biosynthesis and Accumulation of Functional Ingredients in Citrus Fruit Peel: A Promising Strategy for a More Sustainable Crop. J Agric Food Chem. 2021 Nov 24;69(46):13810–20.
40. Sah S, Krishnani S, Singh R. Pseudomonas mediated nutritional and growth promotional activities for sustainable food security. Curr Res Microb Sci. 2021 Dec;2:100084.
41. Mudi L, Muhidin, Rakian TC, Sutariati GAK, Leomo S, Yusuf DN. Effectivity of Pseudomonas fluorescens TBT214 in increasing soybean seed quality in different seed vigor. IOP Conf Ser Earth Environ Sci. 2021 Jul 1;807(4):042069.
42. Chitra P, Jijeesh CM. Biopriming of seeds with plant growth promoting bacteria Pseudomonas fluorescens for better germination and seedling vigour of the East Indian sandalwood. New For (Dordr). 2021 Sep 4;52(5):829–41.
43. Prusty S, Sahoo RK, Sharaya R, Tuteja N, Gill SS. Unraveling the potential of native Azotobacter and Azospirillum spp. formulations for sustainable crop production of rice (Oryza sativa L. var. Khandagiri). South African Journal of Botany. 2023 Nov;162:10–9.

 doi:10.20944/preprints202501.0779.v1

44. Naqqash T, Malik KA, Imran A, Hameed S, Shahid M, Hanif MK, et al. Inoculation With Azospirillum spp. Acts as the Liming Source for Improving Growth and Nitrogen Use Efficiency of Potato. Front Plant Sci. 2022 Jul 28;13.
45. Sumbul A, Ansari RA, Rizvi R, Mahmood I. Azotobacter: A potential bio-fertilizer for soil and plant health management. Saudi J Biol Sci. 2020 Dec;27(12):3634–40.
46. Rocco DHE, Freire BM, Oliveira TJ, Alves PLM, de Oliveira Júnior JM, Batista BL, et al. Bacillus subtilis as an effective tool for bioremediation of lead, copper and cadmium in water. Discover Applied Sciences. 2024 Aug 5;6(8):430.
47. Arce-Inga M, González-Pérez AR, Hernandez-Diaz E, Chuquibala-Checan B, Chavez-Jalk A, Llanos-Gomez KJ, et al. Bioremediation Potential of Native Bacillus sp. Strains as a Sustainable Strategy for Cadmium Accumulation of Theobroma cacao in Amazonas Region. Microorganisms. 2022 Oct 25;10(11):2108.
48. Das A, Das N, Rajkumari J, Pandey P, Pandey P. Exploring the bioremediation potential of Bacillus spp. for sustainable mitigation of hydrocarbon contaminants. Environmental Sustainability. 2024 Apr 30;7(2):135–56.
49. Golnari M, Bahrami N, Milanian Z, Rabbani Khorasgani M, Asadollahi MA, Shafiei R, et al. Isolation and characterization of novel Bacillus strains with superior probiotic potential: comparative analysis and safety evaluation. Sci Rep. 2024 Jan 17;14(1):1457.
50. Hussein MH, Saeed IO. Isolation, Identification Bacteria and Bioremediation of Soil Contaminate Crude Oil from Specific Area (Baiji_Iraq). Journal for Research in Applied Sciences and Biotechnology. 2022 Oct 31;1(4):187–93.