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Advances in Bioresearch

## ORIGINAL ARTICLE

# A study on Bacterial Diversity in the top-most producing iron mines, Bailadila, Chhattisgarh

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**ABSTRACT**
*The growing impact of mining activities on biodiversity has prompted increased attention toward understanding soil microbial communities in mining regions. This study investigates the culturable bacterial diversity in soil samples collected from iron ore mines in Chhattisgarh, India. A total of 64 bacterial isolates were obtained and characterized using morphological, Gram staining, and a range of physiological and biochemical tests. These included assessments for antibiotic resistance, phosphate solubilization, biofilm formation, and indole production. The isolates demonstrated varied morphologies and included both Gram-positive and Gram-negative bacteria. Notably, several isolates showed resistance to multiple antibiotics and positive traits like phosphate solubilizing ability and biofilm production—attributes significant for agricultural and environmental applications such as bioremediation and plant growth promotion. While the isolates exhibit promising biological activities, further molecular characterization, including 16S rRNA gene sequencing, is necessary to confirm their taxonomy and potential functional roles. The findings highlight the importance of microbial diversity studies in mining environments for sustainable ecosystem management and potential biotechnological applications.*
***Keywords:*** *mine, antibiotic-resistance, PGPB (Plant Growth Promoting Bacteria)*

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## INTRODUCTION

The intersection of mining and biodiversity is an increasingly critical area of study as human activities continue to exert pressure on natural ecosystems [37]. Mining operations, while essential for economic development, often lead to habitat destruction, pollution, and the loss of species diversity. These impacts threaten the resilience of ecosystems and undermine conservation efforts globally [24]. Despite growing recognition of the need to address the environmental consequences of mining, significant gaps remain in understanding the long-term effects on biodiversity and the effectiveness of mitigation strategies [37]. Mining activities impact biodiversity in a variety of direct and indirect ways, often leading to significant ecological degradation. The most immediate and obvious impact is habitat destruction, as large-scale excavation, deforestation, and land clearance for mining operations destroy critical habitats for plants and animals [2,11,13,27]. This disruption can lead to the fragmentation of ecosystems, isolating species and reducing genetic diversity. Additionally, mining can result in pollution, with the release of toxic chemicals such as heavy metals, acid mine drainage, and particulate matter, which contaminate soil, water, and air, further threatening the health of surrounding ecosystems [25,28]. Soil biodiversity, particularly the diversity of soil microbes, plays a pivotal role in enhancing both the nutritional quality and safety of food. Healthy soil microbial communities improve nutrient cycling, increasing the bioavailability of essential nutrients like iron, zinc, and vitamins, which are critical for human health [8,26,34]. Additionally, diverse soil microbes help suppress harmful pathogens, reducing the risk of foodborne diseases [5]. Overall, the cumulative impacts of mining activities can significantly diminish biodiversity, challenging efforts to conserve and restore ecosystems. Antibiotics, originally developed for medical use, are now pervasive environmental pollutants due to their widespread use in human

healthcare, animal husbandry, and agriculture [9]. While antibiotics play a crucial role in combating infections, their presence in the environment, primarily through wastewater discharge, agricultural runoff, and livestock waste, has raised significant concerns about their potential ecological effects [7]. The ecological impacts of antibiotics are complex, as these substances can interfere with the natural balance of microbial communities that are vital for ecosystem functioning. Antibiotics can alter microbial diversity, promote the growth of antibiotic-resistant bacteria, and disrupt essential ecological processes such as nutrient cycling and decomposition [17]. Hence, it is essential to study the microbial diversity of the soil in order to identify antibiotics, if present, and subsequent mitigation efforts. Metal toxicity is a significant environmental issue due to the ability of metals to accumulate in living organisms and their resistance to biodegradation [23]. Heavy metals such as aluminium (Al), lead (Pb), cadmium (Cd), gold (Au), mercury (Hg), and silver (Ag) are harmful to living organisms [36]. To address the pollution caused by these toxic metals, bioremediation techniques are used, where microbes [1,31,39] or their enzymes [32] are employed to transform the metals into less harmful forms. Microorganisms employ various strategies to survive and interact with inorganic metals in their environment. These strategies include biotransformation, metal extrusion, enzyme activity, the production of exopolysaccharides (EPS), [12,43] and the synthesis of metallothioneins [23]. To cope with metal toxicity, microbes have evolved a range of mechanisms for metal resistance and detoxification [6]. The study of microbial diversity in mining areas can open new doors for medicinal, industrial, and agricultural applications, including new drug compound formulation, bio-remediation, as well as help in conservation efforts. In this experiment, culturable bacteria were studied through isolation and characterisation. The isolation process involved the collection of microorganisms, preservation, transportation the same and examination through a microscope. The sample was diluted, and the pour plate method was used, which involved pouring agar on a petri dish and then spreading the diluted sample on the petri dish for further observation. Characterisation was done using Gram staining and various morphological and biochemical tests.

## MATERIAL AND METHODS

### Sample collection and study area

Soil samples were collected from an iron mine, which is located in the Dantewada district of Chhattisgarh, India. Five samples were collected randomly from each site, both Bacheli and Kirandul. Ten soil samples were collected into sterile polybags. The samples were stored at 4°C till further use.

### Isolation and purification of bacteria

Bacterial isolates were isolated by the standard serial dilution protocol. Briefly, 1gm of soil was dissolved into a total of 10 ml of 0.9% sterile saline and was properly mixed with the help of a vortex. 1ml of suspended soil was transferred into the next test tube filled with 9 ml of saline water and made $10^{-1}$ dilution, and this step was continued till a $10^{-5}$ dilution. 300µl of $10^{-1}$ to $10^{-5}$ diluted sample was spread on a large (150 mm) nutrient agar plate, in three sets. These plates were incubated at room temperature for 24 hours, and the appearance of different colonies was recorded, on the basis of morphology, texture, etc. Different isolates were selected for further study on the basis of their different physical characteristics. Pure cultures were prepared, and glycerol stocks were kept for long-term storage.

### Gram-staining test

All isolated bacteria were characterized by Gram staining test and microscopic studies. The standard Gram staining protocol was followed, and then prepared slides were observed under a light microscope (OPTIKA microscopes, Italy, B-383PHI). Gram-negative bacteria look pink/ red, while Gram-positive bacteria appear blue/purple. Further test were characterised on selected 33 bacteria only.

### *Physiological and biochemical tests used for the identification and characterisation of isolates*

### Growth in different media

#### *MacConkey agar:*

This media is used as a selective and differential culture medium for the isolation of Gram-negative and enteric bacteria. Pure cultures of isolates were streaked onto prepared MacConkey agar (Himedia) plate and incubated for 48 hours at 37°C. After incubation, isolates grown on MacConkey agar plates were considered Gram-negative bacteria.

#### *Pikovskaya's agar:*

For the test of phosphate solubilizing activity of isolates, we used Pikovskaya medium. Pure cultures of selected isolates were streaked on the prepared Pikovskaya's agar plates and incubated at 37°C for 5 days. The appearances of a clear zone around bacterial growth were considered a positive result for phosphate solubilization activity [42].

***CAS blue agar:***
Selected isolates were grown into CAS blue agar medium plates, which is commonly used for detection of siderophore production in bacteria. Prepared basal media with pH 6.7 and CAS solution separately and then mixed it. Autoclaved it by ratio 9:1 and prepared CAS blue agar media. Pure cultures of selected isolates were streaked on a CAS plate and incubated for 48 hours at 37°C. Then, I observed the plate for 10 days. The appearance of yellow/orange halo zone around the bacterial growth were considered as positive for siderophore production.

***King's B media:***
Selected isolates were grown on King's B medium plates, which is commonly used for isolation of Pseudomonas and for studying fluorescence production. Pure cultures of selected isolates were streaked on prepared King's B media plate and incubated for one week at 37°C, then fluorescence production was observed under UV light (360nm) [40].

***Congo red agar:***
Congo red agar media is used to study the capability to biofilm formation, as described by Freeman et al. (1989). Fresh culture of isolates was streaked into prepared Congo red plates and incubated for 24-48 hours at 37°C. Black colonies with a dry crystalline consistency were considered a positive result [14].

***Disk diffusion assay:***
Standardized protocol of Kirby-Bauer disk diffusion susceptibility test was applied to selected isolates. For this purpose, we used a total of 8 readymade antibiotic disks with known concentration, namely Azithromycin (30mcg), Vancomycin (30mcg), Rifampicin (5mcg), Ampicillin (10mcg), Levofloxacin (5mcg), Streptomycin (25mcg), Gentamicin (10mcg), and Penicillin (10 units). Briefly, 300 µL of overnight-grown culture was spread on prepared nutrient agar plates and incubated for approximately half an hour. After incubation, Antibiotic disks were placed on plates and further incubated for 18-24 hours for 24hours at 37°C. After incubation, a clear zone around the disk shows sensitivity. The zone diameter was measured. No such clear zone indicates resistance against antibiotics [21].

***Biochemical and other tests:***
Biochemical tests of the selected isolates were performed as per the protocols described by Bergey [20]. Important tests, which were performed, are Urease test, Catalase test, Starch-hydrolysis test, Oxidase test, Glucose fermentation test, and motility test. Indole test was also performed according to the protocol given by Maria et al. (2008) for the detection of Indole production [30].

## RESULTS

### Morphological and microscopic studies
On the basis of morphological studies, we isolated a total of 64 bacteria from the iron-mine soil. Named as BB (Bailadila Bacheli) and BK (Bailadila Kirandul). As per the microscopic studies among 64 isolates, 40 bacteria were characterised as Gram-negative and 24 bacteria were characterised as Gram-positive. Bacteria were shaped, varying from Cocci to Bacilli, while their size and shape of the isolates are shown in Table 1. Representative pictures of Gram-negative and Gram-positive bacteria are also shown in Figure 1.

### Growth in different media
Selected isolates were grown in specific media to study their growth behaviour. Some strains showed good growth on all the plates. i.e., MacConkey agar, Pikovskaya's agar, Congo red agar, CAS blue agar, and King's B agar plates. When King's B culture plates were checked for fluorescence under UV light, all of them were negative. 20 bacteria were growing properly on the MacConkey agar plate, and 13 bacteria were not growing properly. so, probably 20 bacteria were Gram-negative and 13 were Gram-positive. 25 bacteria were showing a zone on the Pikovskaya agar plate, and the remaining 8 were not showing a zone around the bacteria. So, 25 bacteria have phosphate solubilizing activity. 27 bacteria showed a yellow or orange halo zone around the bacteria. So, 27 bacteria were capable of siderophore production. Only 12 bacteria produce black pigment on the CRA (Congo-red agar) plate. So, they were capable of biofilm formation. Representative pictures of Agar media assay were shown in Table 2 and Figure 2 and 3.

### Antibiotic disk diffusion assay
Antibiotic resistance profiling of the strains was also done by disk diffusion assay. Results of the disk diffusion assay with different concentrations of antibiotics used against all selected samples, Figure 5. BB 1.11, BB 2.1, BB 2.2, BB 2.3, BB 2.4, BB 3.5, and BK 1.5 showed that these organisms were resistant to Ampicillin. Likewise, BB 1.6, BB 1.7, BB 1.10, BB 1.11, BB 2.1, BB 2.2, BB 2.3, BB 2.4, BB 3.6, were resistant to Penicillin, whereas BB 1.10 and BK 1.3 were resistant to Rifampicin and Streptomycin, respectively. The rest of them are sensitive to other antibiotics shown in Figure 5. Among 8 antibiotics for which the strains are sensitive, Azithromycin had highest impact as shown by largest zone of inhibition and

Rifampicin had lowest impact as was evident from lowest impact as was evident from lowest size of zone inhibition (figure 4).

**Biochemical test:**

Selected isolates were further characterised by various biochemical tests are presented in Table 4 and figure 6. All 33 selected isolates showed positive results for the catalase test. 24 bacteria were motile and 9 were non-motile. All 33 selected isolates were negative results for indole test. 20 bacteria were found positive for oxidase test and 13 were negative. The urease test, indicated that, BB 2.1, BB 2.3, BB 2.5, BB 3.2, and BB 3.8 were changed its colour from yellow to pink i.e. positive and rest were negative remain yellow colour. 15 bacteria were found positive for starch-hydrolysis test and 18 were negative. Based on the carbohydrate fermentation reaction, bacteria are classified as: fermenters with acid production only, fermenters with both acid and gas production and non-fermenting bacteria. Glucose fermentation test indicated that BB 1.1, BB 1.2, BB 1.5, BB 1.7, BB 1.12, BB 2.8, BB 2.9, BB 2.10, BB3.1, BB 3.2, BB 3.5, BB 3.6, BB 3.8 were fermenter with acid production only, while BB 1.10 and BB 2.7 were classified as fermenter with both acid and gas production. Additionally, it is observed that some bacteria showed different colour, which may be a result of some kind of pigment production (Reiner, 2012).

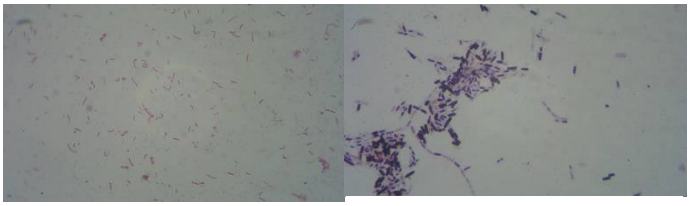



**Figure no. 1: Representative pictures of microscopic observation. a. Gram-positive bacteria (BK 1.2); b. Gram-negative bacteria (BK 1.4)**

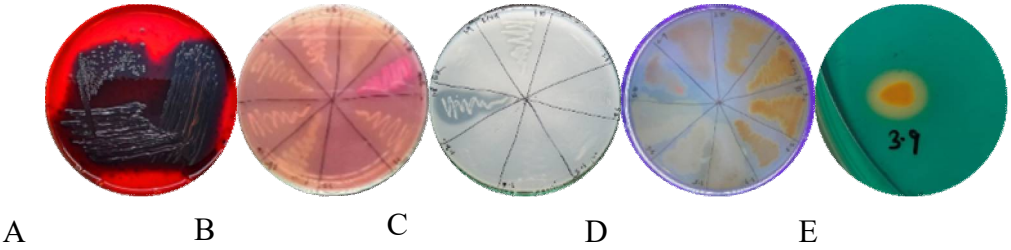



**Figure 2: Representative pictures of different Agar media assays. a. Congo-red agar, b. MacConkey agar, c. Pikovskaya's agar, d. King's B agar under UV light, e. CAS blue agar**

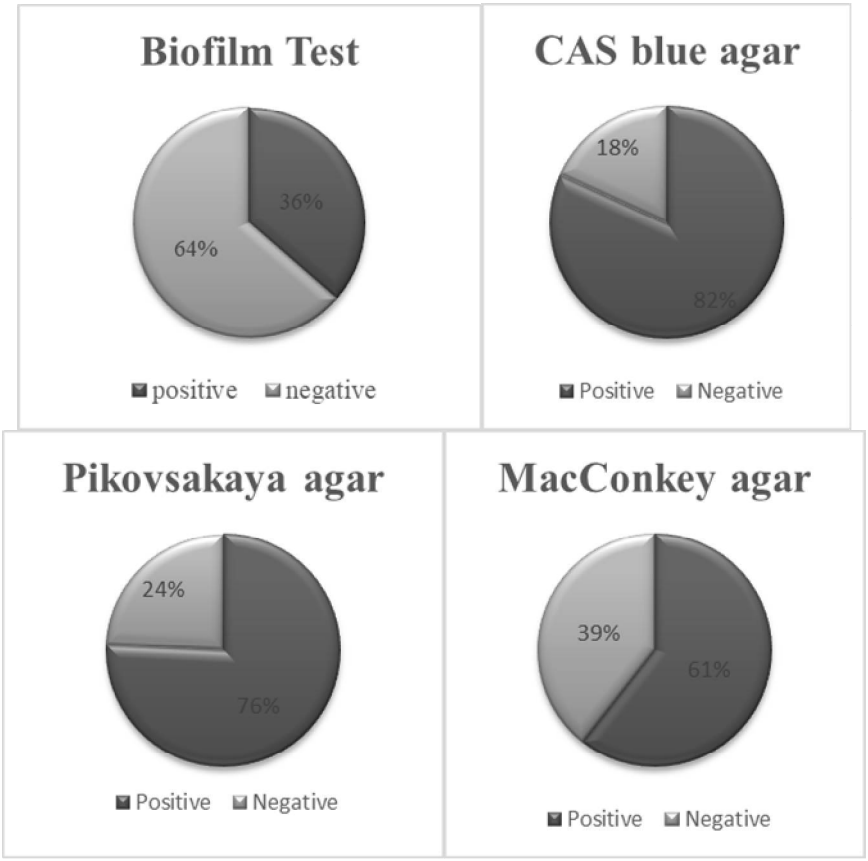



**Figure 3: Graphical representation of Agar Assay Result**

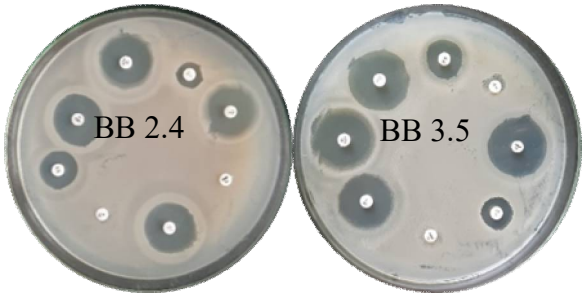



**Figure 4: Representative pictures of the Antibiotic disk diffusion assay.**

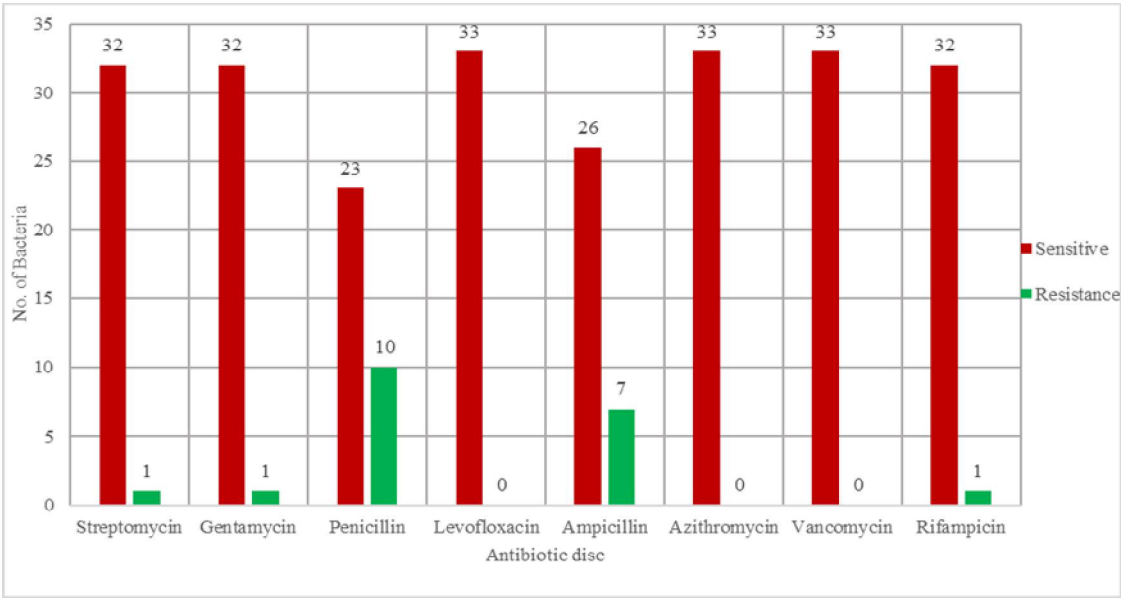
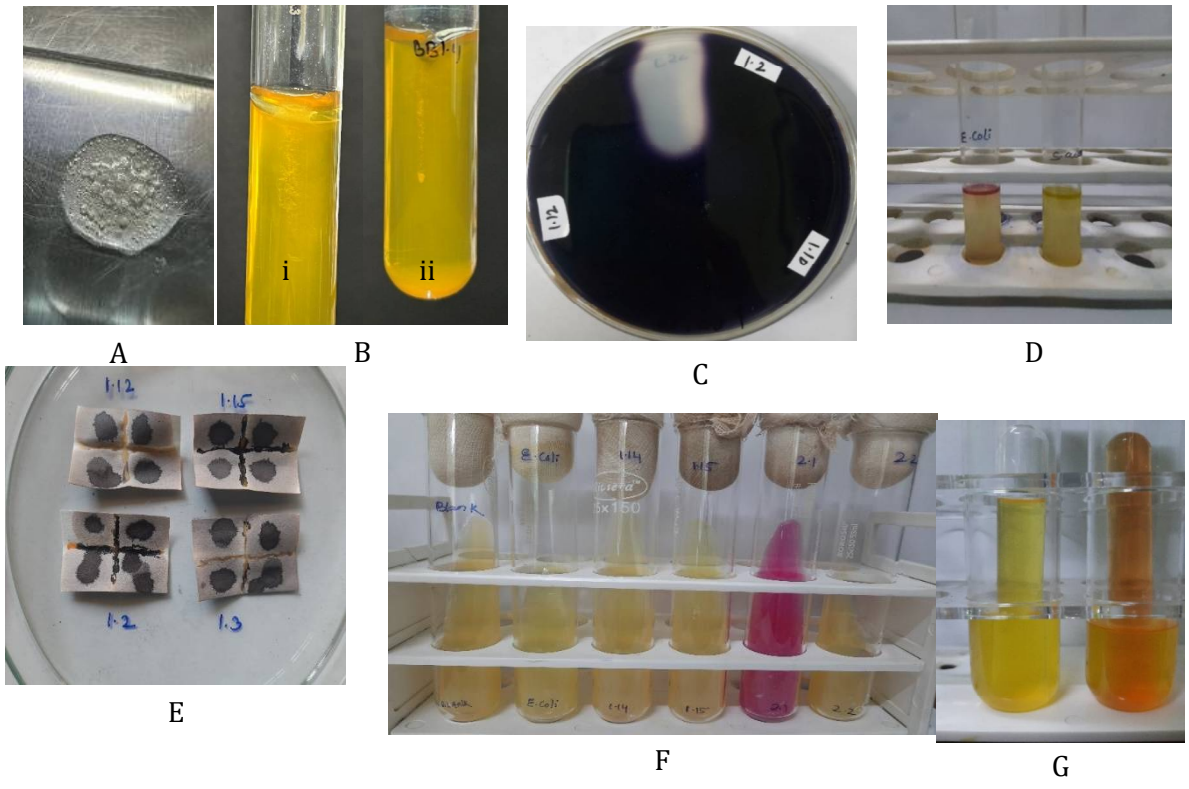



**Figure 5: Summary and results of the Antibiotic disk diffusion assay**




A B C D

E F G

**Figure 6: biochemical and other tests a. catalase test; b. SIM assay i. motile and ii. Non-motile; c. starch hydrolysis test (BB 1.2 showing positive); d. indole test (*E.coli* showing positive as control); e. oxidase test (streaked as plus sign); f. urease test (BB 2.1 showing positive); g. fermentation test (i. positive with gas production, ii. negative)**

**Table no. 1: Microscopic observation**

| S.No. | Sample No. | Gram stain | Shape and size of Bacteria | S.No. | Sample No. | Gram stain | Shape and size of Bacteria |
|---|---|---|---|---|---|---|---|
| 1 | BB 1.1 | Negative | Bacillus | 33 | BB 3.8 | Negative | Streptobacillus |
| 2 | BB 1.2 | Negative | Bacillus | 34 | BB 3.9 | Positive | Streptobacillus |
| 3 | BB 1.3 | Negative | Coccus | 35 | BB 3.10 | Positive | Bacillus, small |
| 4 | BB 1.4 | Positive | Coccus | 36 | Bk 1.1 | Positive | Streptobacillus |
| 5 | BB 1.5 | Positive | Streptobacillus | 37 | Bk 1.2 | positive | Bacillus, small |
| 6 | BB 1.6 | Negative | Streptobacillus | 38 | Bk 1.3 | Negative | Coccus, small |
| 7 | BB 1.7 | Negative | Bacillus | 39 | Bk 1.4 | Negative | Bacillus |
| 8 | BB 1.8 | Negative | Bacillus | 40 | Bk 1.5 | Positive | Streptobacillus |
| 9 | BB 1.9 | Negative | Bacillus | 41 | Bk 1.6 | Negative | Bacillus |
| 10 | BB 1.10 | Positive | Bacillus | 42 | Bk 2.1 | Negative | Bacillus, small |
| 11 | BB 1.11 | Negative | Bacillus | 43 | Bk 2.2 | Negative | Bacillus, small |
| 12 | BB 1.12 | Negative | Bacillus | 44 | Bk 2.3 | Negative | Bacillus, small |
| 13 | BB 1.13 | Negative | Streptobacillus | 45 | Bk 2.4 | Positive | Bacillus |
| 14 | BB 1.14 | Positive | Bacillus | 46 | Bk 2.5 | Positive | Bacillus |
| 15 | BB 1.15 | Positive | Streptobacillus | 47 | Bk 2.6 | Positive | Bacillus |
| 16 | BB 2.1 | Negative | Bacillus | 48 | Bk 2.7 | Negative | Bacillus |
| 17 | BB 2.2 | Negative | Bacillus | 49 | Bk 2.8 | Positive | Streptobacillus |
| 18 | BB 2.3 | Negative | Bacillus | 50 | Bk 3.1 | Negative | Streptobacillus |
| 19 | BB 2.4 | Positive | Bacillus | 51 | Bk 3.2 | Negative | Bacillus |
| 20 | BB 2.5 | Negative | Streptobacillus | 52 | Bk 3.3 | Negative | Bacillus |
| 21 | BB 2.6 | Negative | Bacillus | 53 | Bk 3.4 | Positive | Bacillus |
| 22 | BB 2.7 | Negative | Bacillus | 54 | Bk 3.5 | Negative | coccus |
| 23 | BB 2.8 | Negative | Bacillus | 55 | Bk 3.6 | Positive | Bacillus |
| 24 | BB 2.9 | Negative | Bacillus | 56 | Bk 3.7 | Positive | Bacillus |
| 25 | BB 2.10 | Positive | Bacillus | 57 | Bk 3.8 | Negative | Bacillus |
| 26 | BB 3.1 | Negative | Streptobacillus | 58 | Bk 3.9 | Positive | Bacillus |
| 27 | BB 3.2 | Negative | Streptobacillus | 59 | Bk 3.10 | Negative | Bacillus |
| 28 | BB 3.3 | Positive | Bacillus | 60 | Bk 4.1 | Positive | Bacillus |
| 29 | BB 3.4 | Negative | Streptobacillus | 61 | Bk 4.2 | Negative | Bacillus |
| 30 | BB 3.5 | Positive | Coccus, small | 62 | Bk 4.3 | Negative | Bacillus |
| 31 | BB 3.6 | Negative | Bacillus, small | 63 | Bk 4.4 | Positive | Bacillus |
| 32 | BB 3.7 | Negative | Bacillus, small | 64 | Bk 4.5 | Negative | Bacillus |

**Table 2: Summary and results of selected isolates in different media**

| Sample No. | siderophore production | biofilm formation | Mac-conkey | Pikovsakaya |
|---|---|---|---|---|
| BB1.1 | positive | - | ++ | + |
| BB1.2 | positive | + | + | + |
| BB1.3 | positive | - | + | - |
| BB1.4 | negative | - | + | - |
| BB1.5 | negative | - | +++ | ++ |
| BB1.6 | positive | - | ++ | ++ |
| BB1.7 | negative | - | ++ | + |
| BB1.8 | positive | - | +++ | +++ |
| BB1.9 | positive | - | ++ | + |
| BB1.10 | positive | + | ++++ | ++++ |
| BB1.11 | negative | - | ++ | ++ |
| BB1.12 | positive | + | + | - |
| BB1.14 | positive | - | - | + |
| BB1.15 | negative | - | - | - |
| BB2.1 | positive | - | + | - |
| BB2.2 | positive | + | - | ++ |
| BB2.3 | positive | - | - | +++ |
| BB2.4 | positive | + | - | +++ |
| BB2.5 | negative | + | ++ | ++ |
| BB2.6 | positive | - | + | - |

| | | | | |
|---|---|---|---|---|
| BB2.7 | positive | + | ++ | +++ |
| BB2.8 | positive | + | + | - |
| BB2.9 | positive | - | + | ++ |
| BB2.10 | positive | + | - | - |
| BB3.1 | positive | + | - | +++ |
| BB3.2 | positive | + | + | +++ |
| BB3.3 | positive | - | - | ++ |
| BB3.4 | positive | - | - | +++ |
| BB3.5 | positive | - | - | +++ |
| BB3.6 | positive | - | - | +++ |
| BB3.8 | positive | + | - | +++ |
| BB3.9 | positive | - | - | +++ |
| BB3.10 | positive | - | ++ | ++ |

**Table 3: Summary of results of biochemical test**

| Bacteria name | Urease | starch hydrolysis | oxidase | glucose fermentation (+) with gas (++) | indole | catalase | motility |
|---|---|---|---|---|---|---|---|
| BB1.1 | - | - | - | + | - | + | + |
| BB1.2 | - | + | + | + | - | + | + |
| BB1.3 | - | - | - | - | - | + | + |
| BB1.4 | - | - | - | - | - | + | - |
| BB1.5 | - | + | + | + | - | + | + |
| BB1.6 | - | - | - | + | - | + | + |
| BB1.7 | - | - | + | - | - | + | + |
| BB1.8 | - | - | - | - | - | + | + |
| BB1.9 | - | + | + | - | - | + | + |
| BB1.10 | - | - | - | ++ | - | + | + |
| BB1.11 | - | - | - | - | - | + | + |
| BB1.12 | - | - | - | + | - | + | + |
| BB1.14 | - | - | - | - | - | + | + |
| BB1.15 | - | + | + | - | - | + | + |
| BB2.1 | + | + | + | - | - | + | + |
| BB2.2 | - | + | - | - | - | + | + |
| BB2.3 | + | + | + | - | - | + | + |
| BB2.4 | + | + | + | - | - | + | + |
| BB2.5 | + | + | + | - | - | + | - |
| BB2.6 | - | - | + | - | - | + | - |
| BB2.7 | - | - | - | ++ | - | + | + |
| BB2.8 | - | + | + | + | - | + | - |
| BB2.9 | - | - | + | + | - | + | - |
| BB2.10 | - | - | + | + | - | + | + |
| BB3.1 | - | + | + | + | - | + | - |
| BB3.2 | - | + | + | + | - | + | - |
| BB3.3 | - | - | + | - | - | + | + |
| BB3.4 | - | - | + | - | - | + | + |
| BB3.5 | - | + | + | + | - | + | + |
| BB3.6 | - | + | + | + | - | + | + |
| BB3.8 | + | + | - | + | - | + | - |
| BB3.9 | - | - | + | - | - | + | + |
| BB3.10 | - | - | - | - | - | + | - |

## DISCUSSION AND CONCLUSION

This study represents one of the first efforts to explore culturable bacterial diversity in the iron-ore mining regions of Chhattisgarh, India an area undergoing intensive anthropogenic activity. The isolation of 64 distinct bacterial strains from mine-affected soils reveals that microbial life remains active and potentially adaptive, even under high-stress environmental conditions caused by metal contamination, habitat disruption, and pollution [19]. Among these 64 isolates, 40 isolates were identified as Gram-negative bacteria and 24 were identified as Gram-positive bacteria. The presence of both Gram-positive and Gram-negative bacteria, as identified through Gram staining and morphological analysis, indicates a

diverse microbial community structure. Several isolates exhibited key plant growth-promoting rhizobacteria (PGPR) traits such as phosphate solubilization, indole production, and biofilm formation [18,35]. These characteristics are ecologically significant in nutrient-deficient or contaminated soils and suggest that some native bacteria could be harnessed for biofertilizer development or ecological restoration efforts [16]. Biochemical profiling—including catalase, oxidase, and glucose fermentation tests—further highlighted the metabolic diversity among the isolates. Some strains also exhibited gas or pigment production, indicating unique metabolic pathways or secondary metabolite production with potential pharmaceutical or industrial applications. This study was primarily focused on the identification and initial characterization of culturable bacterial isolates. These bacteria were shown to possess biologically important traits such as antibiotic resistance, biofilm formation, phosphate solubilization, and indole production. Investigating bacterial diversity in iron-ore mine soils is crucial for understanding microbial interactions within disturbed ecosystems and assessing their environmental impact [44]. Our selected isolates showed resistance against 8 different antibiotics. Interestingly, antibiotic resistance was observed in several isolates against a range of antibiotics, including Ampicillin, Penicillin, Levofloxacin and Streptomycin, as well as they are positive for biofilm production, indole formation and phosphate solubilization. Biofilm ensure competitive colonization on the rhizoplane and thereby improve plant growth and health [4]. However, the relationship was not studied. There were two possible locations for antibiotic resistance genes, it may present on Chromosomal DNA or plasmid DNA. Plasmids are facultatively presents in bacteria, so we first perform plasmid isolation, and find out many of the bacteria contains plasmid. Plasmids are appliance for horizontal gene transfer. If Antibiotic Resistance Genes present on plasmid, it can move more frequently to another bacteria (i.e. conjugative plasmid). This resistance may stem from long-term environmental exposure to antibiotic pollutants or horizontal gene transfer among soil microbes [10,29]. While concerning from a public health standpoint, such resistance underscores the evolutionary adaptability of microbial life in disturbed environments. We speculate that regulation of these characteristics may be connected with Horizontal Gene Transfer based on evidences available in literature. Bacterial identification needs to be confirmed by 16S rRNA sequencing. One notable limitation is the reliance on culturable methods, which may overlook a significant proportion of microbial diversity, particularly those adapted to extreme or specific microenvironments. Many microbes remain unculturable using standard techniques, and molecular approaches such as 16S rRNA gene sequencing are necessary for more accurate taxonomic identification and understanding of phylogenetic relationships and functional genes [3,22]. Despite these limitations, the study provides essential baseline data on the microbial ecology of mining-impacted soils. It opens avenues for biotechnological applications, including the use of native microbes in heavy metal bioremediation and enhancement of soil fertility in degraded lands [15]. These findings underscore the importance of conserving microbial biodiversity as a key component of broader ecological and environmental conservation strategies [41].

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