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Isolation and Characterization of Plant Growth Promoting Bacteria from the Rhizosphere of *Chamaecytisus ruthenicus* (Russian broom) Growing on Chalky Soil

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1. **Introduction**

- Intensive use of **agro-chemicals** has negative effect on the environment and animal health (Singh *et al*., 2027).

- Plant growth promoting rhizobacteria (**PGPR**) have often been used as **potential substituent** of Agro-chemicals (Palaniyandi *et al*., 2022)

- PGPR promotes plant growth either through **direct** or **indirect** mechanisms (Khan *et al*., 2022)

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- Some PGPR **directly** promoting plant growth (eg. increase in length of shoot and root). While others **indirectly** promoting plant growth by inhibiting phytopathogens

  (Saharan *et al*., 2011).

- However, the **mechanism** of PGPR activity differ depending on **host plant species**, **soil type**, and **soil nutritional status** (Kalam *et al*., 2021).

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Azospirillum	*Alcaligenes*	*Acinetobacter*
Pseudomonas	*Bacillus*	*Aeromonas*
Klebsiella	*Burkholderia*	*Agrobacterium*
Azotobacter	*Serratia*	*Bradyrhizobium*
Enterobacter	*Herbaspirillum*	*Xanthomonas …*

Bacteria belongs to the listed genera have been reported as effective and efficient PGPR (Sezen et al., 2016).

2. **Statement of the problem**

- Researchers are still looking for **potential PGPR** from the rhizosphere of different plant species.

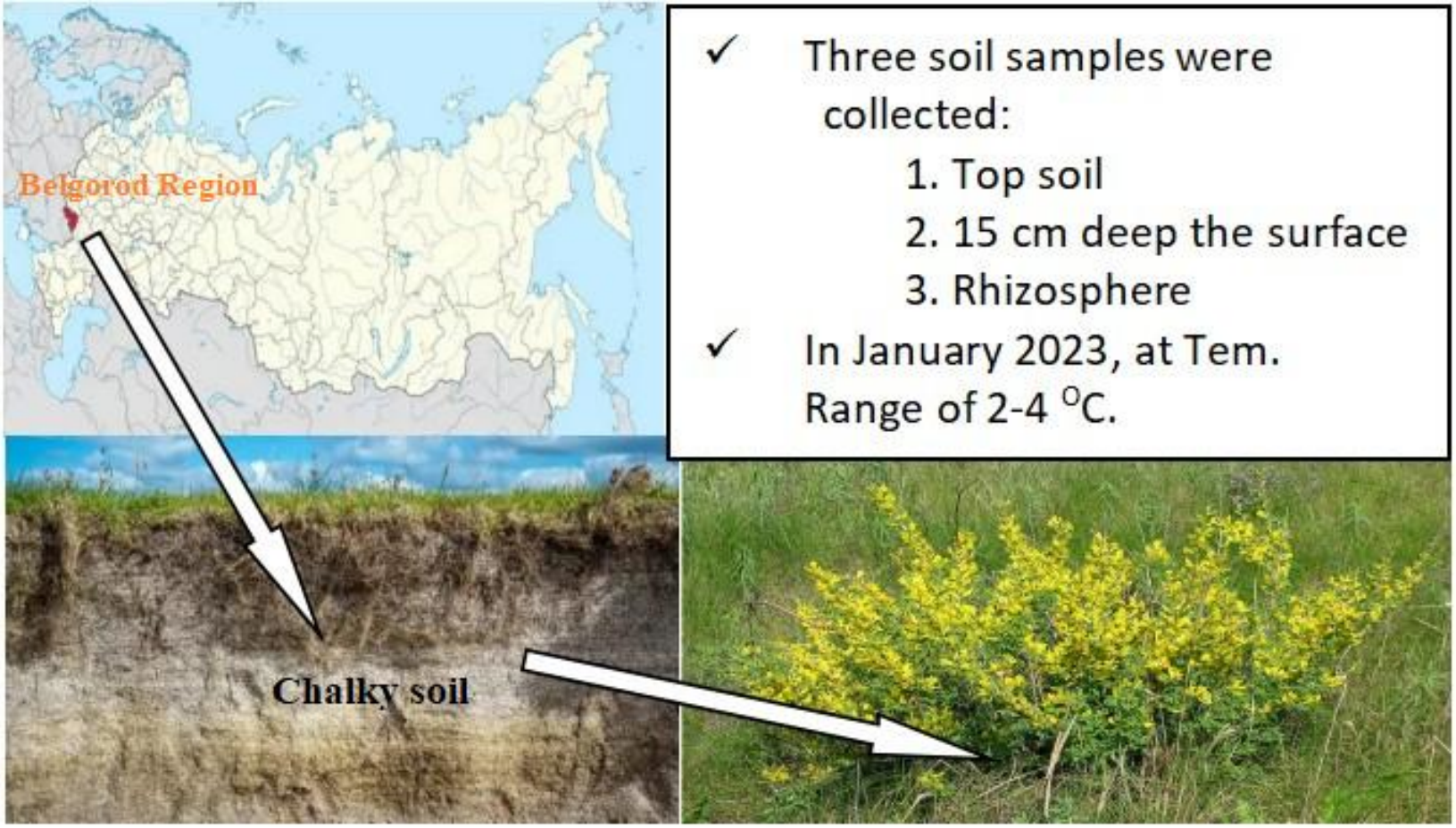
- However, **no research** has been done on chalky soil bacteria found in the rhizosphere of *Chamaecytisus ruthenicus* (**the wild legume plant**).

3. **Objectives**

- Therefore, the aim of the present study was:
  - ✓To **evaluate the abundance of culturable bacteria**
  - ✓To **assess the morphology of bacterial isolates**
  - ✓To **profile chalky soil bacterial community**
  - ✓To **characterize their ability to stimulate plant growth.**

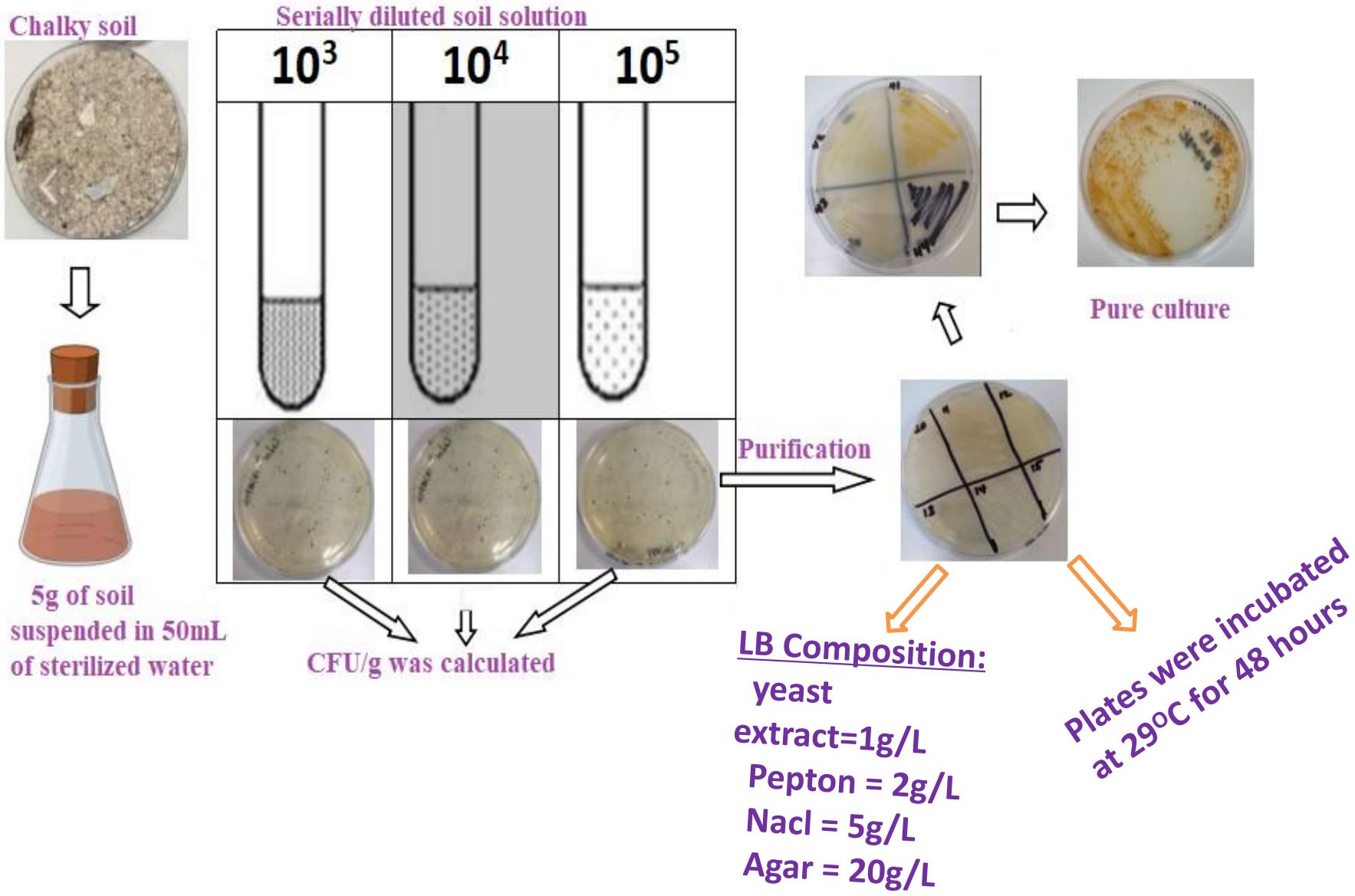
4. Materials and Methods

4.1. Soil sample collection



- ✓ Three soil samples were collected:
  1. Top soil
  2. 15 cm deep the surface
  3. Rhizosphere
- ✓ In January 2023, at Tem. Range of 2-4 ^{O}C.

4.2. Bacterial abundance and isolation



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4.3. **Microscopy examination of bacterial isolates**

- Bacterial cells morphology were obeserved using **light microscop**, after the cells have been fixed with glutaraldehyde solution.

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4.4. Soils profile by 16S rRNA gene sequencing

- Sequence analysis was performed using **QIIME2 v.2022.2 software** (Bollyen *et al*., 2019) and **MicrobiomeAnalyst 2.0 web service** (Dhariwal *et al*., 2017)

4.5. Bacterial inhibition against phytopathogenic microbes

- All the tested bacterial isolates (**Z10, Z11, Z12, Z15, Z26, and Z44**) and **phytopathogenic microbes** (**three** bacteria and **five** fungi) were cultured for **five** and **three** days, respectively.
- **5 µL** of bacterial solution was added onto plates containing phytopathogenic microbes
- Plates were incubated at **29 ^{o}C** for **48** hours.
- Presence of **inhibition zone** indicated inhibition effect.

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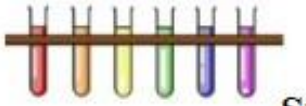
4.6. Bacterial stimulation effect on Plant growth



Six bacterial isolates (Z10, Z11, Z12, Z15, Z26, and Z44) were cultured in 10 mL of LB liquid medium and incubated them at 29 0C for 72 hrs.

15 mL of bacterial solution (OD600=0.1) was added into each plates (total 28 plates)

Germination of seeds (wheat, maize, oats, and lentils) was done under controlled experiment. the control groups not treated with bacterial isolates

The length and fresh weight of shoot and root of the germinated seeds were measured

15 mL of sterilized water is given every day for one week.

5. Results

5.1. Abundance and isolation of rhizospheric bacteria

- The number of **CFU/g**:
  1. Top soil sample = **4.25×10^8** cfu/g
  2. 15 cm beneath the surface = **3.58×10^8** cfu/g
  3. Rhizosphere = **10.1×10^7** cfu/g
- A total of **23 morphologically diversified** chalky soil bacteria were isolated

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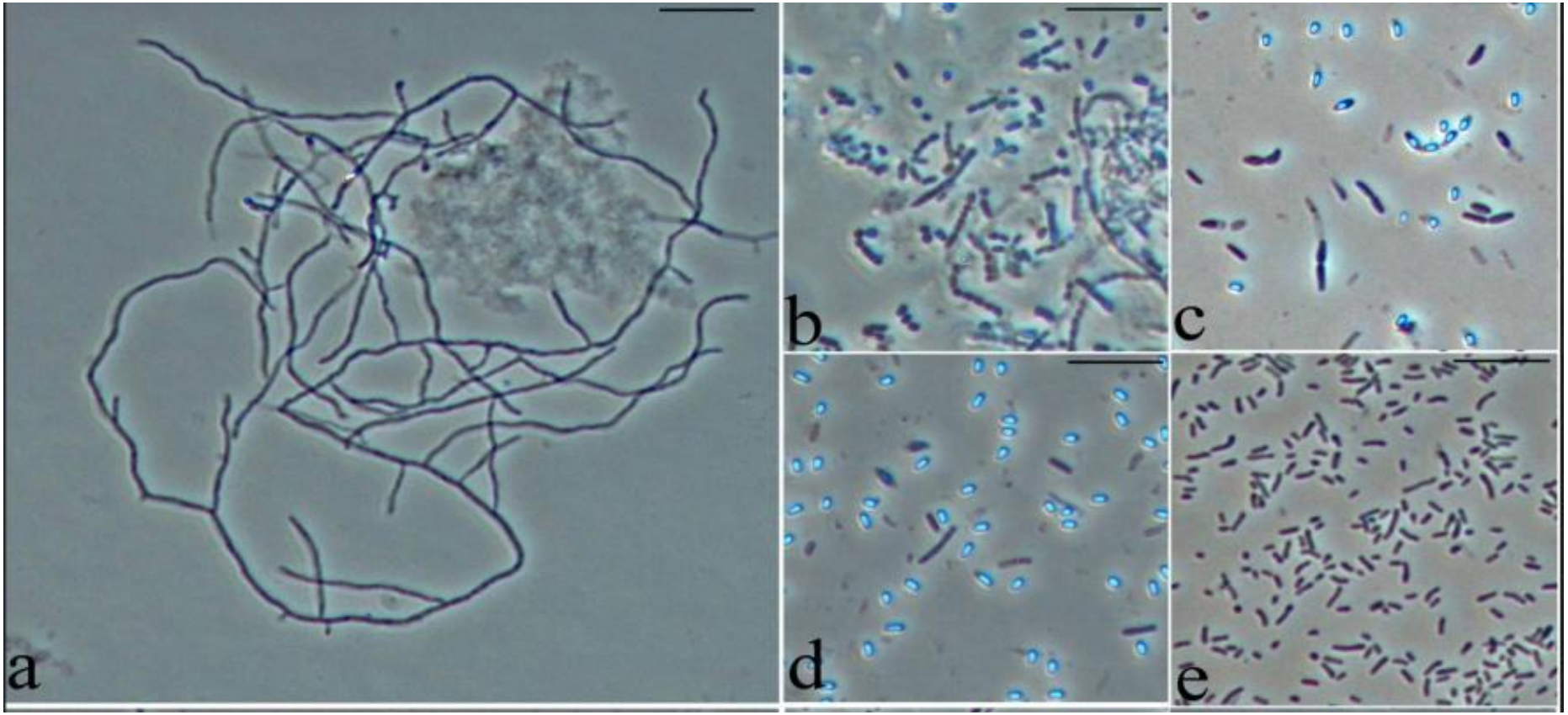
5.2. Morphology of the isolated strains



Chalky soil bacteria with a wide range of morphology

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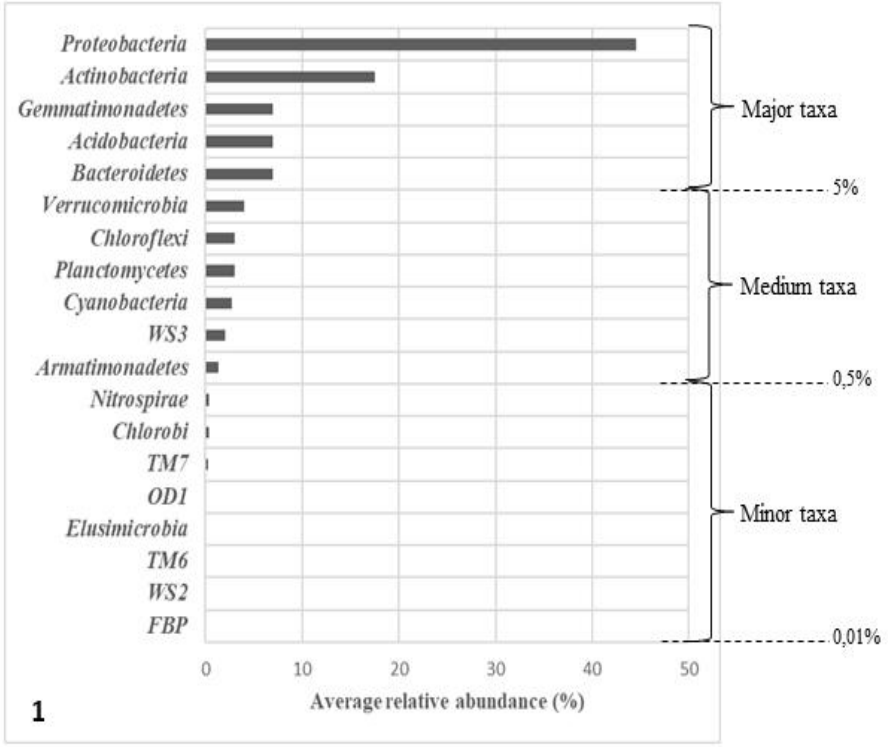
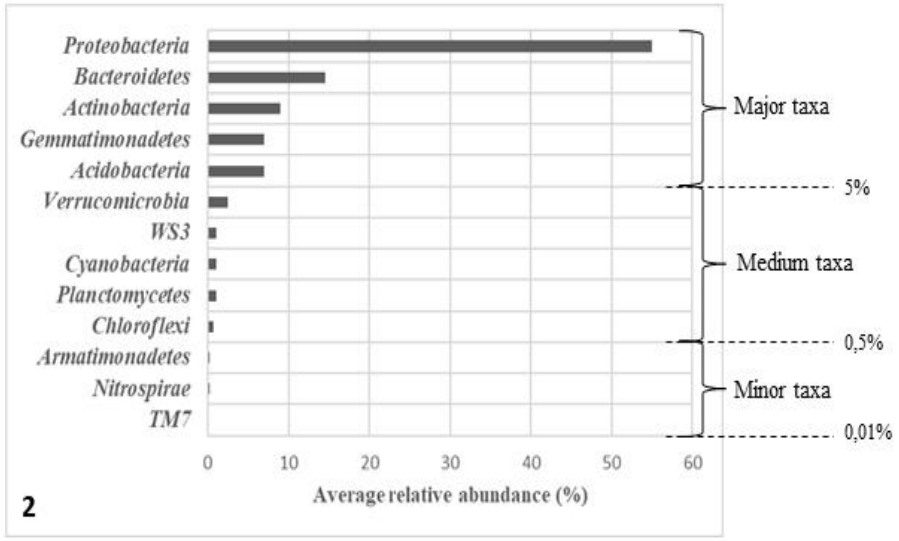
5.3. Profile of soil bacteria community





Proteobacteria **were the most dominant and cosmopolitan group among all the bacterial communities.**

5.4. **Bacterial inhibition against phytopathogenic microbes**

Table 1. Antagonistic test against phytopathogenic microbes

Phytopathogenic Bacteria and Fungi			**Potential PGPR**			
	Z10	**Z11**	**Z12**	**Z15**	**Z26**	**Z44**
Erwinia herbicola ATCC 27155	-	-	-	-	-	-
Micrococcus roseus B1236	-	+	+	+	+	-
Erwinia carotovora B15	-	-	-	-	-	-
Fusarium avenaceum F-132	+	+	+	+	-	+
Rhizoctonia solani F-895	-	+	-	+	-	-
Alternaria brassicicola F-1864	-	+	+	+	-	-
Bipolaris sorokiniana F-4006	+	+	-	+	+	-
Pythium ultimum F-4782	-	+	-	+	-	-

(+) represented growth inhibition (-) represented no growth inhibition

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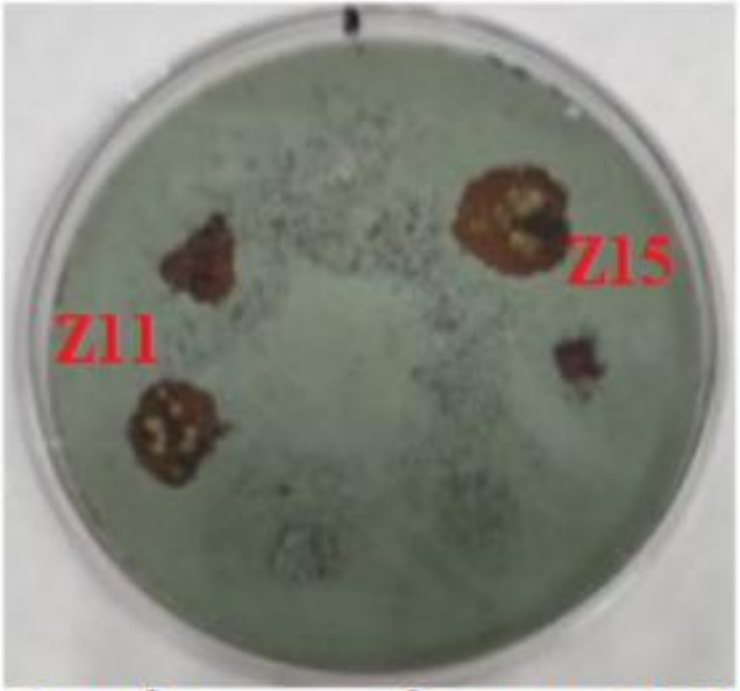
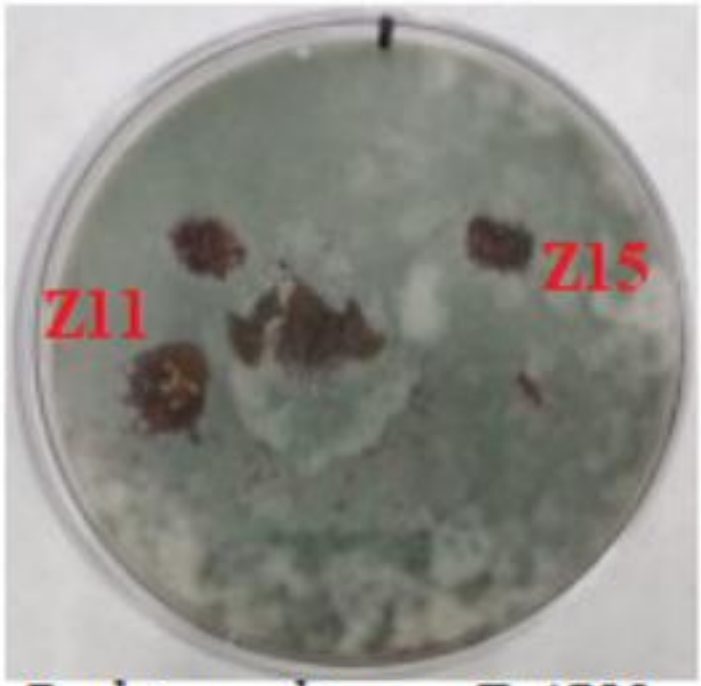


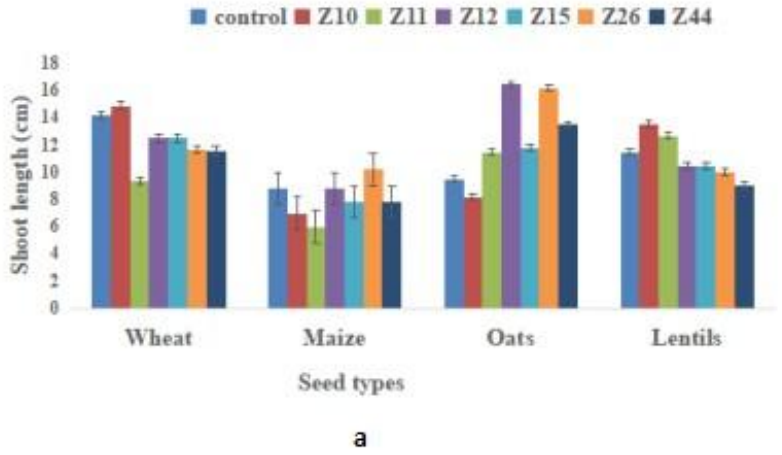
Bipolaris sorokiniana F-4006



Pythium ultimum F-4782

Formation of inhibition zone by bacterial strains Z11 and Z15 against two phytopathogenic fungi

5.5. Bacterial stimulation effect on germinated seeds (wheat, maize, oats, and lentils)



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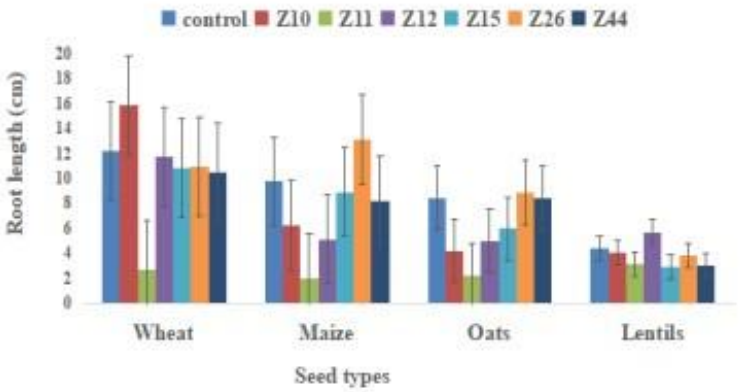

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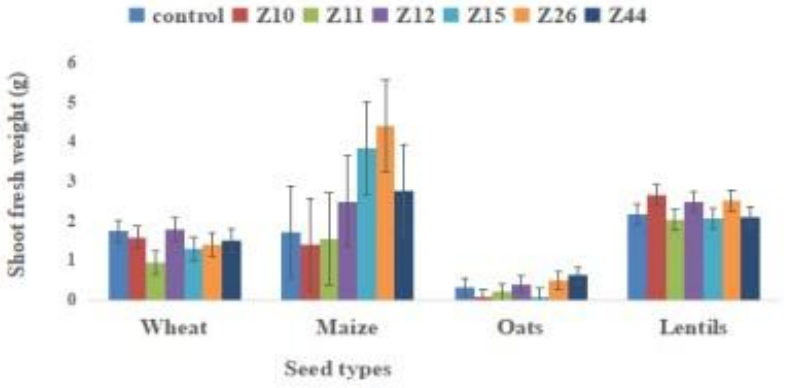

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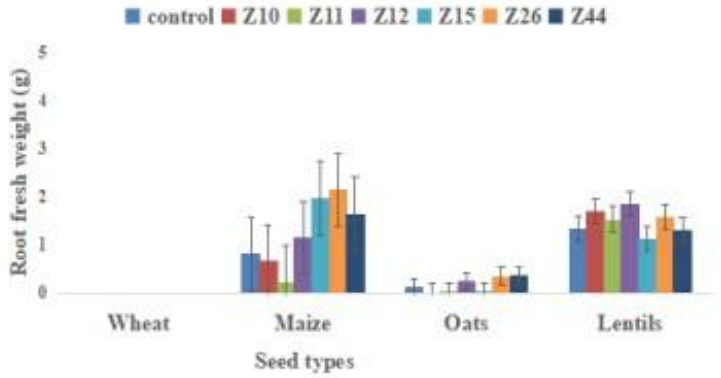

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6. **Conclusion**

- Chalky soil is comprised of **morphological diversified bacterial community**.
- All the selected bacterial isolates showed both **growth stimulation** and **phytopathogenic microbial inhibition activity**.

- This is the **first report** on chalky soil bacteria found in the rhizosphere of wild legume plant.
- In the future, **further investigation** will be carried out on these **potent chalky soil bacteria**.

Thank you !!!!!