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Isolation and Characterisation of Microorganisms from Rhizospheric Soil of Seabuckthorn from Garhwal Region of Uttarakhand

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ABSTRACT

A plant with significant nutritional and therapeutic value, Seabuckthorn (common name: Badrifal and Family: Elaeagnaceae) is recognised for its abundance of bioactive substances, including vitamins, flavonoids, polyphenols, and vital fatty acids, and possesses various health benefits and has the potential to cure diabetes and cardiovascular disorders. Seabuckthorn is a drought-resistant shrub that flourishes in harsh environments, as climate change is a major driver of biodiversity loss. In India, it is primarily found in high altitudes of the Indian Himalayan Region (IHR), such as Uttarakhand, Himachal Pradesh, Sikkim, and Arunachal Pradesh. In the present study, 9 rhizospheric microorganisms have been isolated from a soil sample of Seabuckthorn from Garhwal region of Uttarakhand. Out of 9 isolates, 4 were fungal isolates, 4 were bacterial isolates, and the remaining 1 was an actinobacteria. Furthermore, isolated microbes were investigated for biochemical tests including, catalase, oxidase, siderophore production and phosphate solubilisation assay. The analysis shows that all isolates (bacteria, fungi and actinobacteria) were positive for catalase and oxidase test. The two fungal isolates (GEU_SBT03 and GEU_SBT04) and two bacterial isolates (GEU_SBT05 and GEU_SBT06) were found positive for siderophore production. In addition, three fungal isolates (GEU_SBT01, GEU_SBT02, and GEU_SBT04) and two bacterial isolates (GEU_SBT05 and GEU_SBT06) showed positive response for phosphate solubilisation. These results conclude that isolated microbes (both bacteria and fungi) from the rhizosphere of Seabuckthorn possess plant growth-promoting activity, which can be further applied as biofertilizers for promoting high yield of Seabuckthorn plant and other biotechnological applications.

Keywords: Seabuckthorn; Indian Himalayan region; Rhizosphere; Plant growth-promoting microorganisms

1. INTRODUCTION

Seabuckthorn is a hardy, deciduous, and multipurpose shrub (Fig. 1), widely distributed across Asia and Europe, particularly in cold and arid regions. In recent years, it has attracted considerable scientific and commercial attention owing to its rich phytochemical profile, ecological value, and diverse applications in food, healthcare, and environmental sustainability[1]. Its berries are especially notable for being a rich source of vitamin C (ranging from 275 to over 900 mg per 100 g, far exceeding that of common fruits such as oranges and mangoes), vitamin E (3–30 mg per kg), and B-complex vitamins (including B_1, B_2, B_6, B_{12}),[2] bioactive compounds, including vitamins, flavonoids, carotenoids, and anthocyanins, which impart strong antioxidant properties, boost immunity, and significant therapeutic potential[3]. These unique attributes have positioned Seabuckthorn as a highly sought-after raw material in the nutraceutical and functional food industries.

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In India, Seabuckthorn is predominantly confined to the Indian Himalayan Region (IHR), including the states of Uttarakhand, Himachal Pradesh, Sikkim, and Arunachal Pradesh[4]. The high-altitude soils of Uttarakhand, where the species is found, are typically shallow, coarse in texture, poor in organic matter, and prone to erosion. Climatic extremities such as freezing temperatures, low atmospheric pressure, and high solar radiation further aggravate soil degradation and reduce microbial diversity. Climate change has intensified these challenges, making agriculture and ecosystem stability in such fragile landscapes more vulnerable. Within this context, Seabuckthorn stands out as a climate-resilient species. Its ability to withstand drought, low fertility, and sub-zero temperatures, coupled with a symbiotic association with Frankia bacteria for atmospheric nitrogen fixation, allows it to improve soil fertility, enhance carbon sequestration, and stabilize slopes, thereby directly supporting climate action and land restoration programs in Uttarakhand's mountain ecosystems[5]. Seabuckthorn also serves as a natural shield for fragile landscapes, lowering the risk of landslides in steep mountain areas. Its deep and widespread root system stabilizes loose soils and safeguards fertile topsoil from being washed away during intense rains. Moreover, the plant plays a key role in regulating the hydrological cycle by improving soil water retention within alpine watersheds. Owing to these ecological functions, Seabuckthorn is not only crucial for conserving biodiversity but also for supporting the livelihoods of rural communities in the high-altitude zones of Uttarakhand.

Beyond its nutritional and medicinal value, Seabuckthorn contributes substantially to ecological restoration. Through its symbiotic association with the actinobacterium Frankia in root nodules, the plant is capable of biological nitrogen fixation, which enhances soil fertility and reduces dependency on synthetic fertilizers[6]. Recent estimates show approximately 2.33 million ha of Seabuckthorn worldwide (including wild and cultivated stands), with 2.07–2.10 million ha in China, more recent cultivated-area focused surveys report 918,700 ha globally with an annual production of 400,000 tonnes.[7] Additionally, its extensive root system plays a vital role in soil stabilisation, erosion control, and the reclamation of degraded or marginal lands[6]. Recent research further emphasizes that while Seabuckthorn root nodules harbour a high abundance of Frankia, they exhibit relatively low microbial diversity-a feature closely linked with nodulation specificity and stability, thereby supporting its strong ecological adaptability[8]. In essence, Seabuckthorn fulfils a dual role: it serves as a rich source of nutritionally and pharmaceutically valuable compounds while simultaneously acting as an ecologically significant species for sustainable land management. Its unique ability to bridge human health benefits with environmental resilience highlights the expanding global interest in Seabuckthorn across scientific, agricultural, and industrial domains.

Sati[9], *et al.* also highlighted the role of rhizospheric microorganisms in high-altitude Himalayan plants, demonstrating their contribution to nutrient cycling and stress tolerance under extreme climatic conditions. Furthermore, recent investigations confirm these ecological roles. Ma'murovna[10], *et al.* study shows, successfully isolated rhizospheric microorganisms from Seabuckthorn soils, reinforcing the significance of microbial associations in nutrient cycling and demonstrated that rhizosphere soil properties and antioxidant responses in Seabuckthorn vary with genetically, highlighting functional variability and adaptability in stressed environments. Despite several studies on Seabuckthorn's medicinal potential, fewer reports have focused on microbial communities associated with its rhizosphere in the IHR. The present study aims to isolate and characterize rhizospheric bacteria associated with Seabuckthorn. The objective is to evaluate their plant growth-promoting traits that may contribute to Seabuckthorn's adaptation to high-altitude conditions and further assess their potential for use in sustainable agriculture and soil restoration.

Figure 1. The fruit and plant of seabuckthorn .

2. MATERIALS AND METHODS

2.1 Sample Collection

A rhizospheric soil sample of Seabuckthorn was collected from Khet Langasu, Chamoli District, Uttarakhand (Altitude: 1209 meter above sea level) show in Fig.2. The soil was collected in sterile zip-lock bags and stored in refrigerator at 4 ^{o}C and examined within 24-48 hrs of sampling.

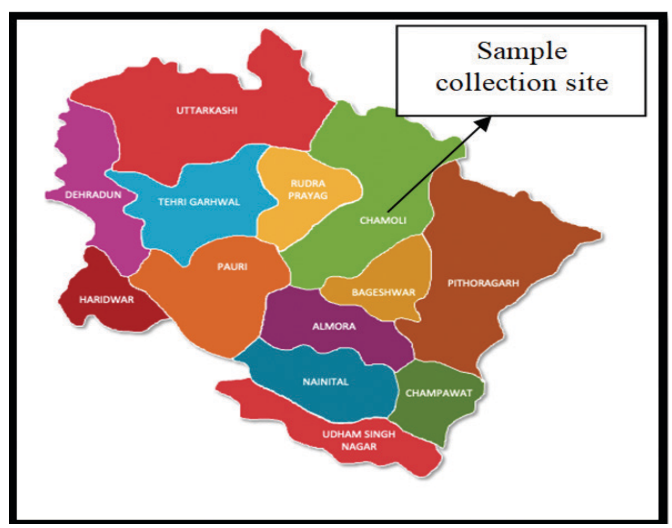

Figure 2. Study site (Khet Langasu, Chamoli district, Uttrakhand).

2.2 Isolation of Microorganisms

Soil sample was sieved and analysed for the isolation of rhizospheric microorganisms. 1 gm of rhizospheric soil was added in 9 ml of distilled water and dissolved homogenously with the help of a vortex shaker. Furthermore, ten-fold serial dilution (10^{-1} to 10^{-10}) was performed for the isolation of microbial colonies. Three media were used for the isolation of rhizospheric microbes such as Nutrient Agar Medium (NAM: SRL company), Potato Dextrose Agar (PDA: SRL company), and Actinomycetes Isolation Agar (AIA: SRL company). 100µl aliquot was suspended on the medium plates for the enumeration of soil microbes and incubated at 25 °C for 2-5 days[1].

2.3 Classification of Microorganisms by Using Staining Method

To identify their morphology based on their size, shape, arrangement, and colour Gram's staining was performed for bacteria and actinobacteria colonies and for fungus lactophenol cotton blue staining. The colonies were observed under Nikon Digital Sight 1000 microscope at 100X magnification.

2.4 Biochemical and PGP's Test

2.4.1 Catalase Test

This method is convenient in examining the presence of catalase enzyme activity in microbial isolates. It eases the release of oxygen from hydrogen peroxide. A loopful of segregated colonies was stowed on a sterile glass slide and few drops of hydrogen peroxide were added on. Formation of bubbles in the test shows positive results in catalase test[12].

2.4.2 Oxidase Test

This test is used to check the presence of the enzyme, cytochrome C oxidase in bacterial cells. A microbe accommodating the enzyme oxidises the reduced colourless reagent. A loopful of each segregated colony was spread on the oxidase discs (HiMedia company). A dark blue tint was obtained from positive results, while negative results showed no colour change[13].

2.4.3 Phosphate Solubilisation Test

The isolates were examined for their phosphate solubilising potential using Gupta *et al.,* method[14]. Isolates were inoculated on modified Pikovskaya's agar (SRL company) with tricalcium phosphate plates and kept at 28±2 °C for 3-5days. The positive colonies showed clear zone surrounding them. The test was determined by solubilisation index: ratio of total diameter (colony+ halozone) and the diameter of colony[15].

2.4.4 Siderophore Test

The obtained colonies were point inoculated on siderophore agar to detect whether the microbes in the colonies conducted siderophore production. Siderophore are iron chelating compounds emanated by microbes that assisted in absorption of iron from their adjacent region. Prepare CAS agar plates using Schwyn & Neilands[16] with some modification. 60.5mg CAS (SRL company) dye dissolved in 50ml DH_2O (solution a), 1mM anhydrous $FeCl_3$ (SRL company) and 10mM HCl (solution b), and 40 mg CTAB in 40ml DH_2O (solution c). Prepare NAM/ King's B media in a conical flax and mix solution a, b, and c in separate conical flask and autoclave them. After the autoclaving let them cool down and mix CAS media and Agar media inside the laminar air flow chamber. Isolates was inoculated on CAS agar plate and kept at 28±2 °C for 24-48 hrs. This would result in colour change from greenish/blue to orange around the extremity of colonies.

3. RESULTS AND DISCUSSION

The results revealed a diverse range of characteristics and activities among the isolated microorganisms obtained from serial dilution. Isolation of rhizospheric microbes was carried out using different medium i.e. NAM, PDA, and AIA. The highest population was found on 10^{-3} CFU. Furthermore, pure cultures have been streaked on agar plates and were identified based on their colour, shape, size, and arrangement by performing Gram's staining and the lactophenol cotton blue staining method. Furthermore, 4 fungi, 4 bacteria, and 1 actinobacteria have been isolated from the rhizospheric soil as observed in Fig. 3.

The isolates found in low-temperature environments help the plant to survive under low-temperature stress condition that impacts their biochemical elements. However, all isolates were positive for catalase and oxidase activity given in Table 1. For siderophore production, 2 fungal isolate, GEU_SBT03 (17mm) and GEU_SBT04 (1mm) and 2 bacterial isolate GEU_SBT05 (15mm) and GEU_ SBT06 (5mm), show a positive response Fig. 4. However, out of 9 isolates, 3 fungal and 2 bacterial isolates demonstrated positive activity against the phosphate solubilisation test. The maximum activity with 13mm zone was determined in GEU_SBT06 isolate followed by GEU_SBT05, GEU_SBT04, and the least activity in GEU_SBT02 and GEU_SBT01 with 4mm, 4mm, 2mm, and 2mm zones, respectively shown in Fig. 4.

In higher altitudinal regions, residence of the soil ecosystem produces the biological breakdown of organic compounds, which enhances the absorption in the host plant[17]. The plant microbiome associations with the soil have been characterised for their plant growth-promoting as well as antagonistic properties in several habitats of the high altitudes region[18]. The pressure of increased population has increased the supply demand of food and emphasised the uses of chemical substances to enhance the production and yield but on the other hand it will drastically declining the quality of food. In addition, the applications of rhizospheric microorganisms have the potential to use as a bio-fertiliser which gives benefits to agricultural practices[19-20]. However, Seabuckthorn also contributes in Sustainable Development Godals (SDSs) via

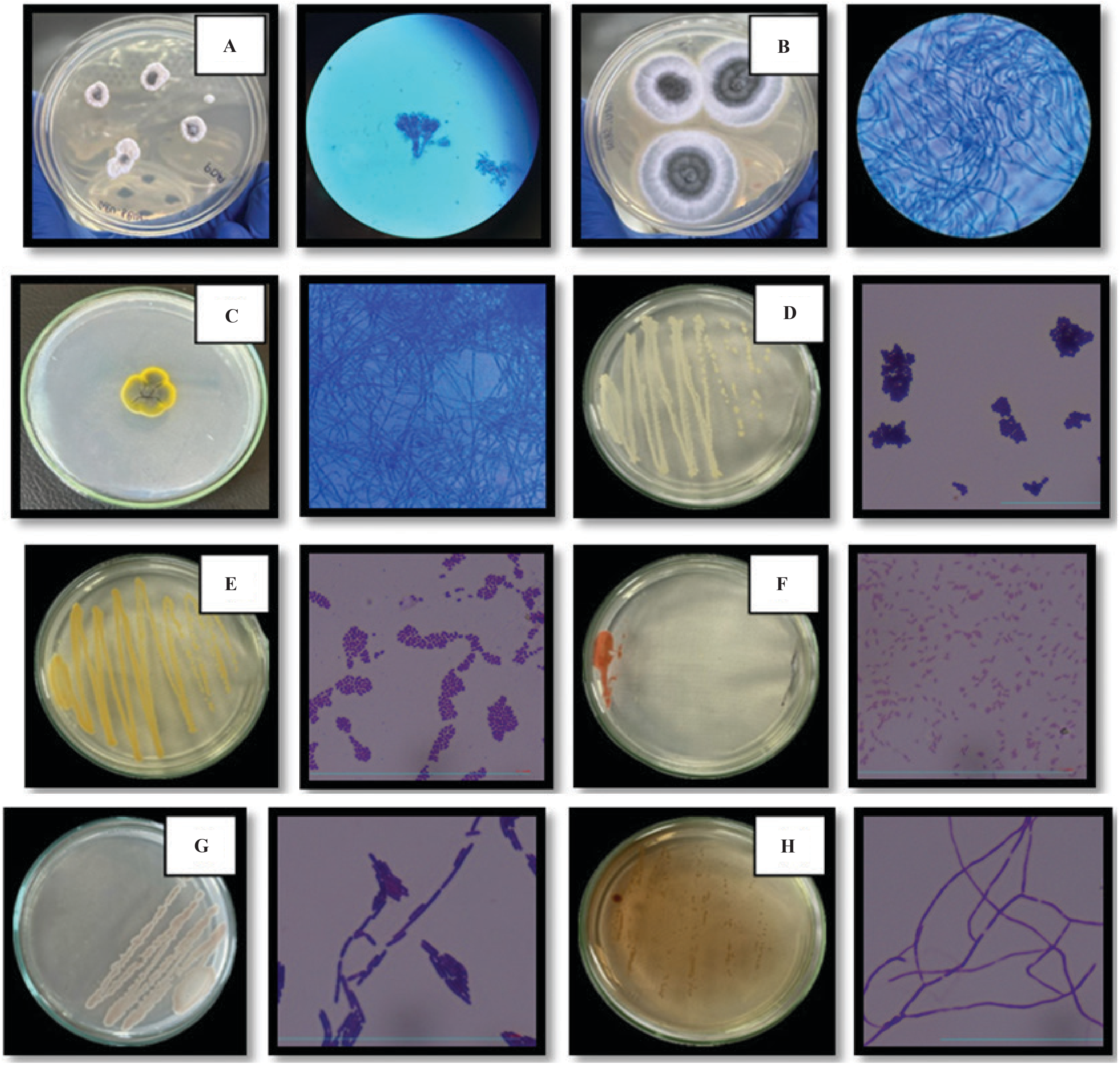

Figure 3. Morphological characterisation of rhizospheric microorganisms (A-C fungi, (D-G) bacteria, and (H) actinobacteria).

Table 1. Biochemical and plant growth promotion characteristics of the isolates from the rhizosphere of Seabuckthorn

Cultural type	Isolates	Catalase	Oxidase	Siderophore production	Phosphate solubilisation
Fungi	GEU_SBT01	+	+	-	+
	GEU_SBT02	+	+	-	+
	GEU_SBT03	+	+	+	-
	GEU_SBT04	+	+	+	+
Bacteria	GEU_SBT05	+	+	+	+
	GEU_SBT06	+	+	+	+
	GEU_SBT07	+	+	-	-
	GEU_SBT08	+	+	-	-
Actinobacteria	GEU_SBT09	+	+	-	-

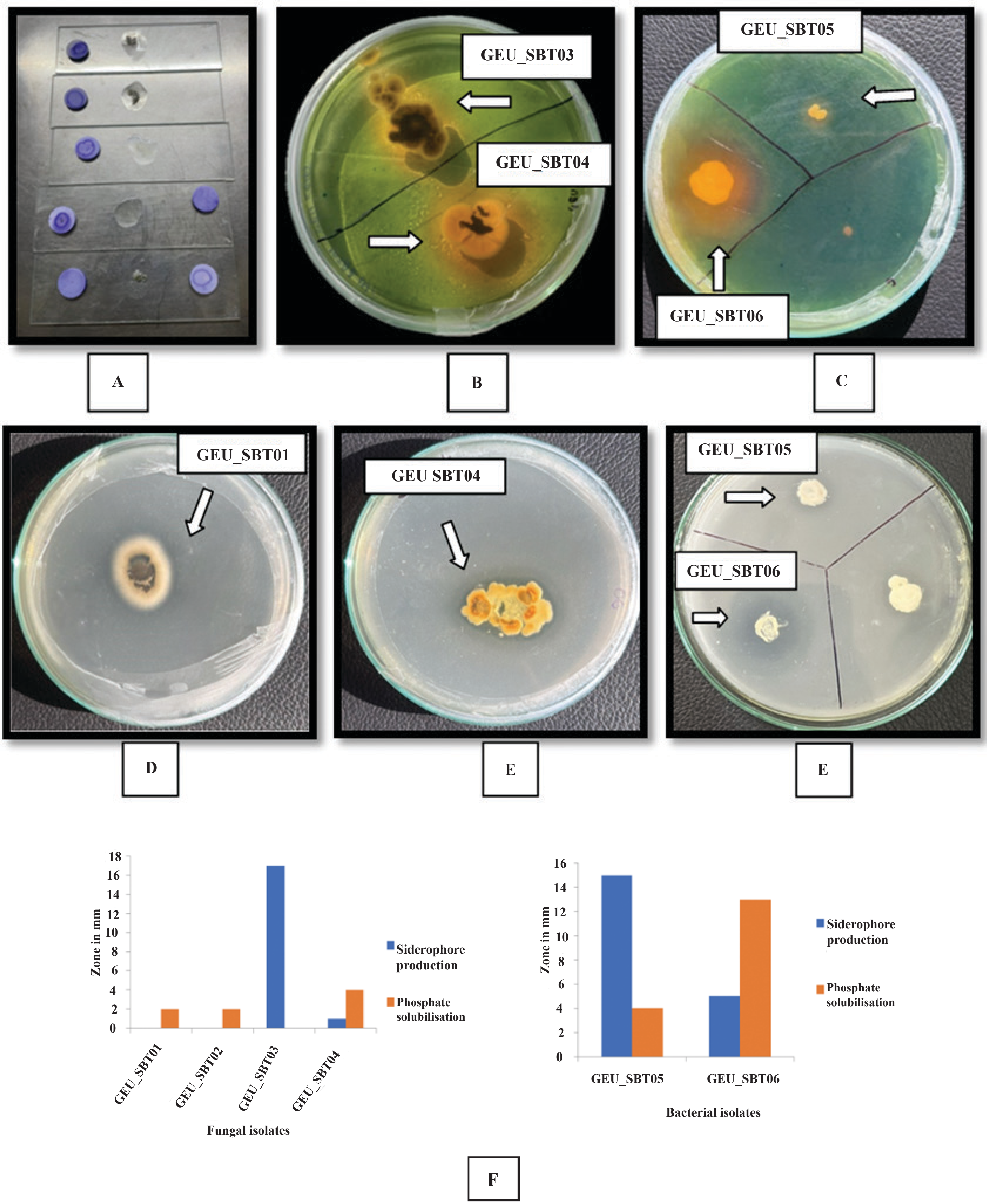

Figure 4. Biochemical and PGP test of isolated microorganisms. (A) catalase and oxidase test, (B-C) siderophore production, (D-E) phosphate solubilization, and (F) qualitative estimation of siderophore test and phosphate solubilisation.

various factors such as economic, environment, nutritional, and social benefits[21]. The plant mainly contributes in SDG-2 (zero hunger), SDG-3 (good health and well-being), SDG-8 (decent work and economic growth), SDG-13 (Climate action), and SDG-15 (life on Land).[22] As its berry in the rich source of vitamins, omega fatty acids, and antioxidants which has potential benefits for skin, cardiovascular disorder, and boost immunity in humans. Furthermore, the rhizospheric microorganisms support the plant health, improve the soil fertility, and helps in nitrogen-fixation[1]. It survives in harsh environment like cold-dessert, supports biodiversity, and contributes in sustainable development and livelihood[23].

4. CONCLUSION

The results of the study indicate the significant importance of soil ecosystems and their capabilities to support plant growth and the importance of isolates. The isolated microbes improve soil fertility, enhance the nutrient cycling, and overall health of Seabuckthorn. The siderophore producing microorganisms are highly prevalent and essential for iron acquisition, these results point to a substantial diversity of microorganisms. Phosphate solubilisation varies among the isolates, indicating variations in their metabolic capabilities. Overall, the findings show that these microbes have a variety of useful characteristics that could be useful in microbial ecology, bioremediation, and agriculture. Furthermore, the identification and application of these isolates as precise functions and possible advantages for sustainable agricultural practices is needed.

5. ACKNOWLEDGEMENT

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