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Exploring Actinobacteria Isolation and Screening from Soil Samples

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Abstract

This research paper explores the diversity and potential of Actinobacteria as sources of bioactive compounds through the isolation as well as distinct screening of these microorganisms from different samples of soil. Samples were collected from five geographically distinct sites of Uttarakhand region, and a combination of selective isolation and morphological characterization was done. On the basis of Morphological and Biochemical characterization 21 bacterial strains were isolated and confirmed their identity as members of the Actinobacteria class. Based on the result generated all the strains were found differ in morphological characteristics and affinity towards pigmentation.

Keywords: Strain, Pigment, Actinobacteria, Characterization, Bioactive.

INTRODUCTION

Actinobacteria, one of the largest phyla of bacteria, play a prominent role in various ecosystems, notably in soil where they take part in important processes such as the decomposition of organic matter and the cycling of nutrients [1]. The ability of actinobacteria to produce a diverse array of bioactive secondary metabolites, such as antibiotics, antifungals, immune-suppressants, and antitumor agents, has earned them a special place of prominence [2]. These properties have positioned them as one of the most prolific sources of clinically useful drugs and the interest in exploring this group of bacteria has intensified over the years. Despite their global prevalence and metabolic versatility, Actinobacteria are still considered underexplored, particularly those residing in soil. The richness and complexity of soil environments make them a promising venue for the isolation of an unknown Actinobacteria strains and associated biologically active compounds [3]. Many of these strains remain uncultured and their potential unexplored due to the inherent difficulties in isolating and culturing them in laboratory conditions. This underscores the importance of developing effective isolation and culturing techniques to uncover the potential of these soil-dwelling bacteria. In addition to exploring the diversity of Actinobacteria, also one of our goals is to evaluate their capacity to produce bioactive compounds. Because many pathogens are becoming resistant to the antibiotics currently in use, there is an immediate demand for the discovery of new antimicrobial agents [4]. Soil Actinobacteria, given their prolific production of secondary metabolites, are among the most promising candidates for this task. By employing selective isolation procedures, morphological characterization, and molecular identification techniques, we anticipate the discovery of diverse Actinobacteria species, some of which may have been previously overlooked in conventional isolation procedures [5,6].

This study aims to isolate and screen Actinobacteria from various soil samples, providing a snapshot of their diversity and potential to produce bioactive compounds.

METHODOLOGY

Sample Collection

Soil specimens were gathered from diverse locales spanning the geographical terrains of Uttarakhand, including Tehri-Garhwal, Chamoli, Srinagar, Uttarkashi, and Haridwar. Collection involved scooping from the top 20 cm layer using sterilized tools, and the samples were then preserved in sterile plastic bags for transportation. Upon arrival at the laboratory, the samples underwent air-drying, homogenization, and sieving through a 2mm mesh to eliminate any debris before subsequent procedures [7].

Isolation of Actinobacteria

The soil samples were subjected to pretreatment with dry heat at 120°C for 30 minutes to reduce contamination by other fast-growing bacteria and fungi. Following pretreatment, a serial dilution method was used to isolate Actinobacteria. The soil suspensions were spread-plated onto selective agar media (Starch Casein Nitrate Agar) supplemented with specific antibiotics to inhibit the growth of other microorganisms. The plates were incubated at 28°C for 2-3 weeks to allow the growth of Actinobacteria [7,8].

Morphological and Biochemical Characterization

Isolated colonies were observed for distinct morphological characteristics such as color, texture, and form. Tests such as the Gram staining, catalase test, and oxidase test were carried out by the established protocols to provide a biochemical characterization. In addition, carbon source utilization tests were also carried out to identify their metabolic capabilities [9].

Reproductive Structure Surface

The scanning electron microscope should be utilized to determine the spore's morphology and the surface options available to it. This can be accomplished by using the cultures prepared for examination using the crisscross pattern and a light microscope. After the electron grid has been thoroughly cleaned, a layer of adhesive tape should be applied to the top surface of the grid. After the mature spores of the strain have been carefully placed on the surface of the adhesive tape, a gold coating needs to be applied for half an hour before the specimen can be examined under an electron microscope at various magnification levels. The silhouettes of the reproductive structures can be distinguished by their spiny, smooth, warty, and hairy qualities [10,11].

RESULTS AND DISCUSSION

The soil samples collected exhibited a high level of microbial diversity, as evidenced by the successful isolation of 21 distinct strains of Actinobacteria. Each of the mentioned strains showed particular morphological features. The colonies displayed diverse hues, encompassing shades such as white, orange, pink, yellow, and grey. They also showed diverse textures, with some colonies appearing smooth and shiny, while others were rough or powdery. Morphological variations were also observed in the form of the colonies, which ranged from circular to irregular [14,15]. Overall, these morphological characteristics indicated a wide diversity of Actinobacteria present in the soil samples. The successful isolation of 21 diverse Actinobacteria isolates from various soil samples substantiates the rich microbial diversity of the sampled sites. These results corroborate the findings of earlier studies like good fellow and Williams (1983) that highlighted the significant Actinobacteria diversity in soil ecosystems [1,16]. The morphological variations observed among the isolates, evident in different colony colors, forms, and textures, further attest to the rich biodiversity of Actinobacteria in these soil environments [12,13]. Remarkably, the isolated strains

demonstrated significant antimicrobial activity against one or more indicator microorganisms. This demonstrates that soil Actinobacteria have the potential to serve as a source of unique antimicrobials. aligning with the assertions made in past research (Bull, 2005). The broad-spectrum activity exhibited by three of our isolates against MCP pesticide is particularly noteworthy and warrants further investigation [2,16].

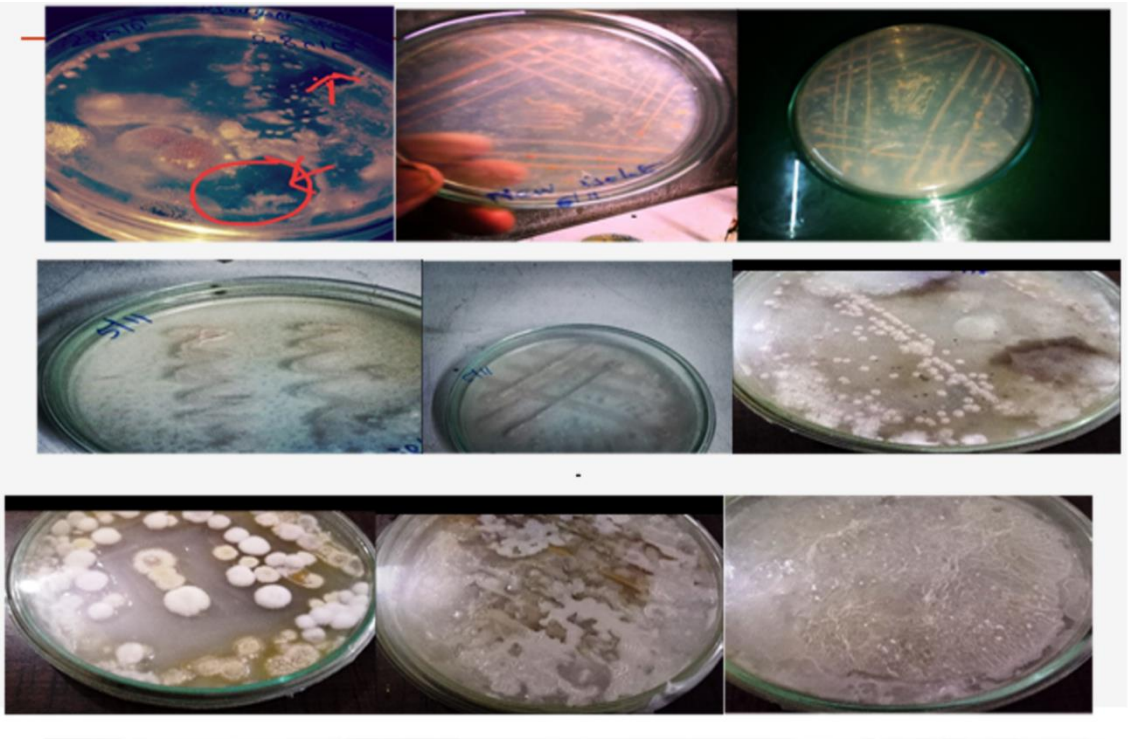

Fig 1. (a)

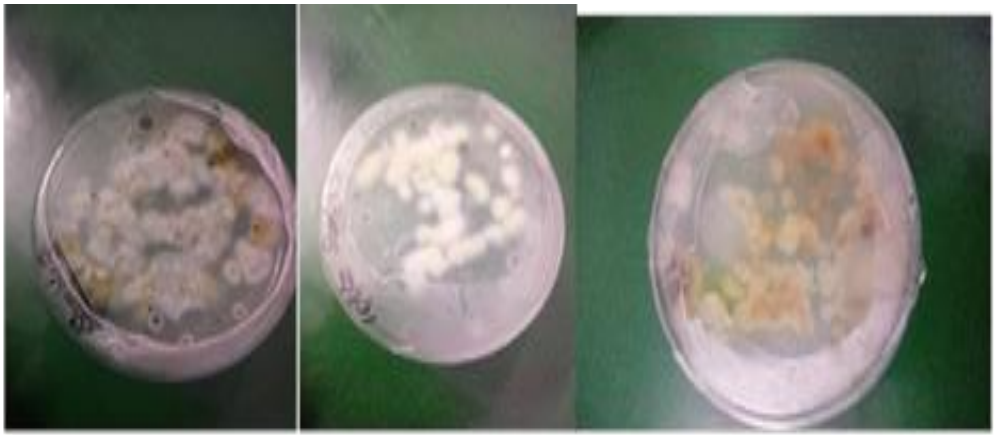

Fig 1. (b)

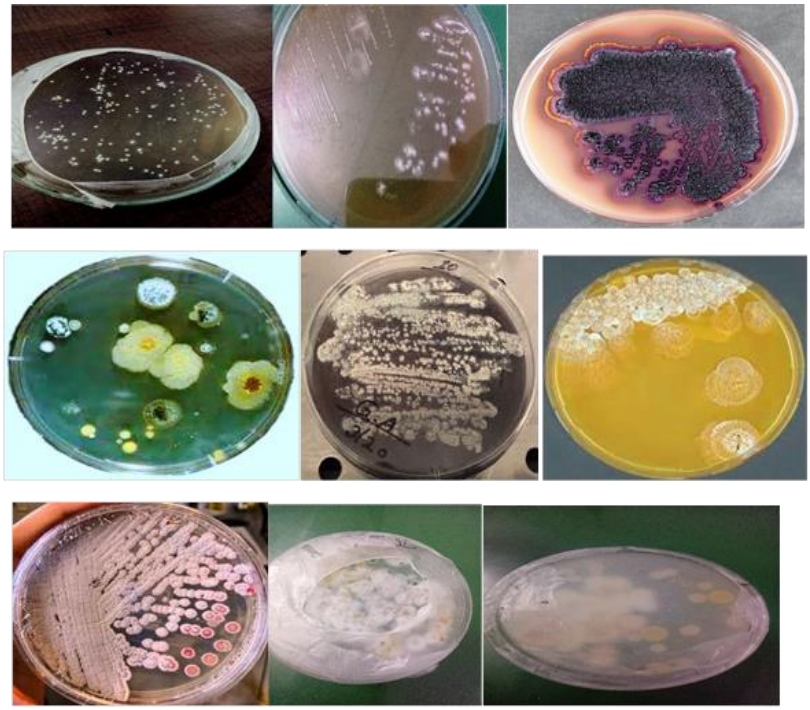

Fig 1. (c)

Figure 1. (a), (b), (c), Actinobacterial isolates as isolated on Y1M6 starch-casein agar medium (1-21) **Each plate showing single isolate; Numbering from stain 1 to strain 21.

The findings of this study bear several important implications. First, they underscore the need for ongoing exploration of soil ecosystems, which remain a reservoir of untapped microbial diversity [19]. With the current crisis of antibiotic resistance, the need for new antimicrobial agents is greater than ever. Our study suggests that soil Actinobacteria could be a promising source in this regard [17]. Furthermore, our results highlight the importance of using a combination of morphological, biochemical, as well as methods based on molecular biology to accurately characterise and identify Actinobacteria. The combination of these methods not only ensures accurate identification but also provides comprehensive insight into the metabolic potential of these microorganisms [18,20].

Table 1. Screening of isolated antibacterial strains based on the Biochemical Characteristics

S.no.	Strain No.	Catalase Activity	Casein Hydrolysis	H_2S Production	Indole Production	Motility	Citrate degradation

1.	ASUK03	++	++	_	-	+	-
2.	ASUK07	++	++	_	-	+	-
3.	ASUK254	++	+	_	-	+	-
4.	ASUK145	+++	+++	_	-	+	-
5.	ASUK67	+++	++	_	-	+	-
6.	ASUK86	++	++	+	-	_	-
7.	ASUK46	++	++	+	-	_	-
8.	ASUK34	+++	+++	_	-	_	-
9.	ASUK23	++	++	_	-	+	-
10	ASUK60	++	++	_	-	+	-
11	ASUK79	+++	+++	_	-	-	-
12	ASUK224	+++	+++	_	-	+	-
13	ASUK185	+++	++	_	-	+	-
14	ASUK145	+++	+++	+	-	_	-
15	ASUK76	+++	+	_	-	+	-
16	ASUK216	+++	+++	_	-	+	-
17	ASUK237	+++	++	_	-	_	-
18	ASUK259	+++	++	+	-	+	-
19	ASUK263	+++	++	_	-	+	-
20	ASUK283	+++	+	_	-	+	-
21	ASUK292	+++	+	_	-	+	-

CONCLUSION

The study provides valuable insights into the diversity and bioactive potential of Actinobacteria in soil. The findings of this research have practical implications in the realm of biotechnology and health sciences. New antimicrobial agents have become crucial in light of the increasing antibiotic-

resistance crisis. Our results suggest that soil Actinobacteria hold considerable potential in this regard, warranting further exploration and exploitation. In terms of research that will be carried out in the future, it would be beneficial to investigate the bioactive potential of the isolated strains of Actinobacteria in greater depth. Studies could focus on purifying and characterizing the antimicrobial compounds produced by these isolates. Furthermore, investigations into the genetic basis of their antimicrobial activity could provide valuable insights into their mechanisms of action. Also, extending this study to include soil samples from more diverse geographical regions could help us better understand the soil Actinobacteria's global distribution and diversity. In summary, our results reveal a high diversity of Actinobacteria in the soil samples, and their potential as a source of novel bioactive compounds. This diversity, combined with their bioactive potential, underscores the significance of Actinobacteria in soil ecosystems as well as the biotech and pharmaceutical use that could be made of them.

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