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# Growth and pigment production by actinomycetes on various media: A comparative study

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***ABSTRACT***
*This study investigates the growth characteristics and pigment production of seven Actinomycetes strains (Act 1, Act 2, Act 3, Act 4, Act 5, Act 6, Act 7and Act 8) on four different media: Nutrient Agar, Starch Casein Agar, Bennet's Agar, and Actinomycetes Agar. The objective was to determine the optimal conditions for the proliferation and pigment synthesis of these strains. Growth parameters assessed included the nature of aerial mycelium, substrate mycelium, and diffusible pigments.*
*Results indicated significant variation in growth and pigment production across different media. Nutrient Agar generally supported poor to moderate growth, with limited pigment production, suggesting suboptimal nutrient content for most strains. Starch Casein Agar and Bennet's Agar facilitated good growth and enhanced pigment production, particularly for strains Act 1, Act 2, Act 5, and Act 6. Actinomycetes Agar showed moderate support for growth but was less effective for pigment synthesis.*
*Notably, Act 1 (Pink1) and Act 2 (Pink II) exhibited robust growth and distinct pigment production on Starch Casein Agar and Bennet's Agar, with Act 2 producing vivid red pigments. Act 4 (Yellow) consistently produced yellow pigments across all media, indicating stable metabolic activity. Strains Act 3 (D. Pink) and Act 7 (Green II) showed limited growth on Nutrient Agar and Actinomycetes Agar but performed better on specialized media.*
*This comparative study underscores the importance of selecting appropriate media to optimize the growth and pigment production of Actinomycetes. The findings offer valuable insights for biotechnological applications, where tailored nutrient environments are crucial for maximizing the potential of these strains. Future research should focus on elucidating specific nutrient and environmental conditions that enhance the metabolic outputs of Actinomycetes.*
***KEYWORDS -*** *pigment production, comparative study, Actinomycetes, different media*

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## I. INTRODUCTION

The ability to produce microbial bioactive compounds makes actinobacteria one of the most explored microbes among prokaryotes. The secondary metabolites of actinobacteria are known for their role in various physiological, cellular, and biological processes.

Actinomycetes population has been known as soil inhabitants. It was stated that only 10% of the actinomycetes have been isolated from nature. So, the researchers need to screen more actinomycetes that have not been discovered and are capable of producing new antibiotics active against bacteria that are resistant to current antibiotics.

Several groups of actinomycetes are stable in bulk soil and rhizosphere plants. Actinomycetes are very important for many plants, where rhizospheric streptomycetes can protect plant roots by inhibiting the growth of fungal pathogen a character based on their ability to produce antifungal antibiotics in vitro. Due to their metabolic diversity, actinomycetes are a good source of lytic enzymes, antibiotics, and other bioactive metabolites. Actinomycetes are free-living Gram-positive bacteria having high G+C content (>55%) in DNA (Kämpfer, 2012). The most important and dominant genus within Actinobacteria is Streptomyces (Ceylan, Okmen, Ugur, 2008). Members of this group are referred to as the biological antagonistic types. They are of special interest since streptomycetes are the ones that are exploited and their metabolites are used in the manufacture of antibiotics (Kekuda, Shobha, Onkarappa, 2010). Streptomyces provides more than half (70%) of the naturally occurring antibiotics (Bérdy, 2005) with high commercial value and continues to be routinely screened for interesting bioactive substances (Takahashi, 2004; Meena et al., 2013).

The actinomycines belong to a family of chromopeptide lactone antibiotics that present antitumor and cytotoxic properties (Praveen et al., 2008). They represent an important class of natural products that, despite being discovered more than 70 years ago, continue to be a focus of many research areas, especially in the biological and medicinal sciences (Kurosawa et al., 2006). Among the actinomycines, actinomycin D has been

studied most extensively and it is used for the treatment of malignant tumors, such as Wilms' tumor (Green, 1977), and childhood rhabdomyosarcoma (Womer, 1997). The biological activity of actinomycin D is related to its ability to bind to the DNA duplex, these being associated with DNA functionality, leading to RNA and, consequently, protein synthesis inhibition (Martinez, Chacon-Garcia, 2005; Boer, Canals, Coll, 2009). The two main mechanisms are intercalation to DNA and the stabilization of cleavable complexes of topoisomerases I and II with DNA, or the drug penetrates to a place in the DNA structure where topoisomerase binds with DNA, respectively (Koba, Konopa, 2005). Actinomycin binds to the highest-energy beta-DNA form found within the boundaries connecting double-stranded B-DNA with single-stranded DNA in the transcription complex (Sobell, 2016) and physically obstructs the transcriptional complex (Huang et al., 2000).

Actinomycin D is produced by a range of Streptomyces species as part of a mixture of actinomycetes (Kurosawa et al., 2006; Praveen et al., 2008).

Exploring new habitats is one of the most promising ways of isolating actinomycete producers of antibiotics endowed with antimicrobial activity (Zitouni et al., 2005; Khanna, Solanki, Lal, 2011; Wadetwar, Patil, 2013). Thus, we report here the antimicrobial activity of actinomycetes isolated from a soil sample collected in Karoo, South Africa and the characterization and identification of actinomycin-producing strain KRG-1

## II. MATERIALS AND METHODS

**Sample collection and processing**

Soil samples (rhizosphere and surface soil) were collected, from the cotton fields of (Latitude 19.113898° Longitude 75.976675°) At. Nathapur Ta & district, Beed-Maharashtra. The mapping of the sampling sites was done by GPS MAP camera.

Soil samples were collected from different localities of Maharashtra State i.e. Beed District areas from the agricultural fields. The exact localities of collected soil samples were given along with the date of collection, field number, and their GPS readings.

For this, we collected rhizosphere soil from cotton crop fields in various regions. The soil collected near the vicinity of the rhizosphere was accounted as the bulk soil. A total of soil samples were collected aseptically in sterile polypropylene bags and were immediately transported to the laboratory for microbiological analysis.

**Isolation of actinomycetes and maintenance –**

One gram of soil sample was taken and serially diluted up to $10^{-10}$ using distilled water as a diluent. The mixture was shaken vigorously using a vortex; 0.1 ml of each dilution was placed on starch casein agar (composition: soluble starch: 10 g, $K_2HPO_4$: 2 g, $KNO_3$: 2 g, casein: 0.3 g, $MgSO_4.7H_2O$: 0.05 g, $CaCO_3$: 0.02 g, $FeSO_4.7H_2O$: 0.01 g, agar: 15 g, and filtered seawater: 1000 ml and pH: 7.0 ± 0.1), and the inoculum was spread properly using a sterile glass spreader.

The inoculated plates were allowed to stand at room temperature for 5–10 minutes to allow the liquid to be absorbed and were incubated at 28°C for 7 days.

| Strain | Nutrient Agar | Starch casein | Bennet's agar | Actinomycetes agar |
|---|---|---|---|---|
| **Act 1 (Pink1)**<br>Growth<br>Arial mycelium<br>Substrate mycelium<br>Diffusible pigment | <br>Poor<br>White<br>White<br>No pigment | <br>Good<br>White<br>Brownish<br>Pink | <br>Good<br>White<br>Off white<br>No pigment | <br>Good<br>White<br>White<br>No pigment |
| **Act 2 (Pink II)**<br>Growth<br>Arial mycelium<br>Substrate mycelium<br>Diffusible pigment | <br>Moderate<br>White<br>Pink<br>Slight pink | <br>Good<br>White<br>Orange<br>Pink | <br>Good<br>White<br>Red<br>Red | <br>Moderate<br>White<br>White<br>No pigment |
| **Act 3 (D.Pink)**<br>Growth<br>Arial mycelium<br>Substrate mycelium<br>Diffusible pigment | <br>No Growth | <br>Moderate<br>Slight brown<br>Brown<br>Not diffusible | <br>Poor<br>White<br>Red<br>Not diffusible | <br>Poor<br>White<br>White<br>No pigment |
| **Act 4 (Yellow)**<br>Growth<br>Arial mycelium<br>Substrate mycelium<br>Diffusible pigment | <br>Moderate<br>White<br>Yellow<br>Yellow | <br>Good<br>White<br>Yellow<br>Yellow | <br>Moderate<br>White<br>Yellow<br>Yellow | <br>Moderate<br>White<br>Yellow<br>Yellow |
| **Act 5 (Beet)**<br>Growth | <br>Moderate<br>White | <br>Good<br>White | <br>Good<br>White | <br>Good<br>White |

| | | | | |
|---|---|---|---|---|
| Arial mycelium Substrate mycelium Diffusible pigment | Off white<br>No | Red<br>Red | Orange<br>Orange | Slight yellow<br>Yellow |
| **Act 6 (Green I)**<br>Growth<br>Arial mycelium<br>Substrate mycelium<br>Diffusible pigment | Poor<br>White<br>Off white<br>No | Good<br>Green<br>Green<br>Not diffusible | Moderate<br>White<br>Yellow<br>Not diffusible | Moderate<br>White<br>White<br>No pigment |
| **Act 7 (Green II)**<br>Growth<br>Arial mycelium Substrate mycelium Diffusible pigment | No Growth | Good<br>Green<br>Green<br>Not diffusible | Moderate<br>Green<br>Green<br>Not diffusible | No growth |
| **Act 8 (Lemon)**<br>Growth<br>Arial mycelium<br>Substrate mycelium<br>Diffusible pigment | Moderate<br>White<br>Lemon<br>Lemon | Good<br>White<br>Lemon<br>Lemon | No Growth | No Growth |

**Growth of actinomycetes on different media and their pigment production**

Growth characters of actinomycetes were studied for 7 days at 28oC using 4 different culture media at pH 7. The compositions of the culture media used are as follows,

1. **Starch casein agar (SCA) (g/l):** Starch, 10.0; Casein, 1.0; CaCo3, 0.02; FeSO4, 0.01; KNO3, 2.0; 1000 ml of distilled water and Agar, 20.
2. **Nutrient agar (NA) (g/l):** beef extract, 1 g; Peptone, 5 g; Sodium chloride (NaCl), 5 g; 1000 ml of distilled water and Agar, 20. 0
3. **Bennet's agar (BA) (g/l):** Tryptone,10; Yeast extract, 5; NaCl,10; MgSO4. anhydrous0.98; Agar15; Final pH7.0 ± 0.2 at 25°C
4. **Actinomycetes Agar (AC) (g/l):** L-asparagine, 0.1 g/L;dipotassium phosphate, 0.5 g/L; ferrous sulfate, 0.001 g/L; magnesium sulfate, 0.1 g/L;sodium caseinate, 2 g/L,sodium propionate, 4 g/L.

## III. RESULTS AND DISCUSSION

Different media have been suggested for the isolation of actinomycetes from the soil, SCA, NA, BA, and Actinomycetes agar were selected to see their efficacy for the growth and production of different soluble pigments. Eight strains of actinomycetes were isolated from soil samples of different habitats grown on different media as mentioned in Fig. 1.

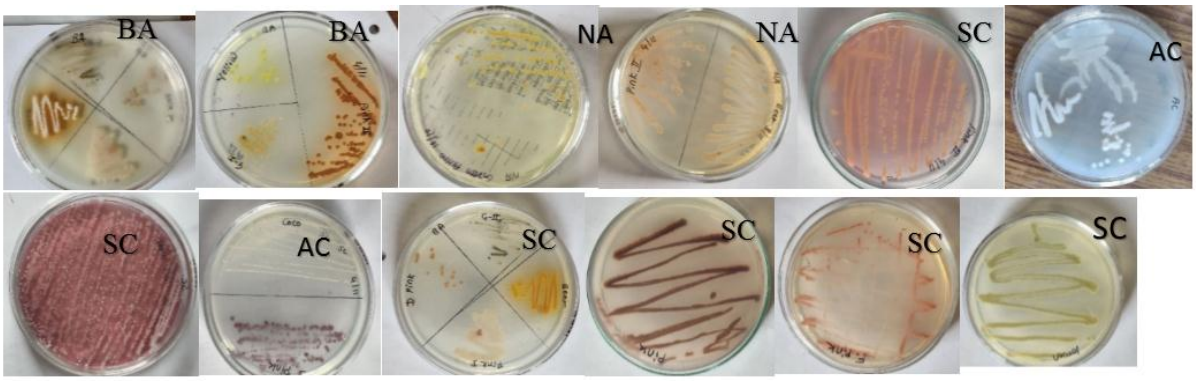


**Fig. 2: Diffusion of pigments in SCA, AA, BA, and NA.**

Growth was best observed on SCA, AA, BA, and NA media at 37°C. On SCA and other media, Act5, and Act 2, produced different diffusion pigments (Table 1 & 2; Fig.2).

The presence of soluble colors other than melanin pigmentation was determined. Actinomycetes are a group of productive sources of secondary metabolites and by far most of these compounds are obtained from the single-family Streptomyces. Actinomycetes have demonstrated their significance both biotechnologically as well as industrially. The isolation and characterization of actinomycetes are an important approach to industrially important natural colors. The isolation of actinomycetes from soil has been described by Selvameenal et al. and their potential for pigment-producing ability along with antimicrobial activities have also been shown. Many industries are using natural color-producing actinomycetes. The growth parameters assessed included the nature

of aerial mycelium, substrate mycelium, and diffusible pigments. The results provided significant insights into the optimal growth conditions and distinctive features of each strain.

The study demonstrates that different Actinomycetes strains exhibit varied growth patterns and pigment production based on the type of media used. Starch Casein Agar and Bennet's Agar generally supported better growth and pigment production compared to Nutrient Agar and Actinomycetes Agar. Understanding these growth characteristics is crucial for optimizing the cultivation and utilization of Actinomycetes in various biotechnological applications. Further research could explore the specific nutrients and conditions that enhance growth and pigment production for each strain.

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