EXPLORING ENZYMATIC POTENTIAL OF AQUATIC CHROMOGENIC ACTINOMYCETES

Om V. Adel[1], Zodpe S. N.[2], Sufiya Areeb[3]

[1] *P.G. Student, P. G. Department of Microbiology, Shri Shivaji College, Akola, Maharashtra, India*
[2] *Associate Professor and Head, P. G. Department of Microbiology, Shri Shivaji College, Akola, Maharashtra, India*
[3] *Research Scholar, P. G. Department of Microbiology, Shri Shivaji College, Akola, Maharashtra, India*

ABSTRACT

Actinobacteria are a diverse group of Gram-positive, filamentous microorganisms known for their significant metabolic versatility, pigment production and biotechnological importance. The present study aimed to explore the enzymatic potential of chromogenic *Actinomycetes* isolated from aquatic environments of the Vidarbha region. A total of 40 water samples were collected from various sources including tap water, bore water, rivers, lakes, wells, waterfalls, and hand pumps. Out of these, 18 samples showed positive growth for chromogenic *Actinomycetes*, indicating their widespread distribution in aquatic habitats. The highest frequency of isolates was observed in bore water (33%), followed by tap water (28%), while other sources such as river, lake, waterfall, well, and hand pump showed comparatively lower occurrence. All isolates exhibited good growth on selective media at neutral pH and temperature range of 26–38°C, with pigment production occurring within 6–7 days. Morphological and microscopic analysis confirmed that all isolates were Gram-positive with septate mycelium and showed variations in colony characteristics and pigment color. Biochemical characterization revealed that all isolates were negative for IMViC tests, indicating their non-enteric and strictly aerobic nature. Carbohydrate fermentation studies showed that all isolates fermented glucose with only acid production, while variable results were observed for other sugars. Enzymatic screening demonstrated that 17 out of 18 isolates showed positive amylase activity, and 17 isolates exhibited catalase activity. Protease production was observed in 5 isolates, while the majority showed urease activity except two isolates. All isolates were negative for oxidase activity.

Keyword: *Chromogenic Actinomycetes, Aquatic Environment, Pigment, Enzymatic Potential*

1. INTRODUCTION

Actinobacteriology has attracted growing attention due to the remarkable metabolic diversity and biotechnological importance of Actinobacteria. These Gram-positive, filamentous microorganisms are well known for producing a wide range of bioactive compounds, enzymes, and pigments. Among them, *Streptomyces* represents a dominant and industrially significant genus**.** Although *Actinomycetes* have traditionally been associated with soil, recent studies highlight freshwater ecosystems as promising and underexplored sources of diverse actinobacterial populations. These environments, characterized by fluctuating physicochemical conditions, support metabolically adaptable microorganisms with unique functional properties. In particular, chromogenic *Actinomycetes*, capable of producing visible pigments, have gained attention due to their ecological adaptability and potential to synthesize bioactive compounds with antimicrobial and antioxidant properties. In addition to pigment production, *Actinomycetes* are recognized for their ability to produce a variety of extracellular enzymes, including Amylases, Proteases, Catalase, Oxidase and Urease. These enzymes play a key role in the degradation of complex organic substrates and are widely utilized in industrial processes. Notably, aquatic *Actinomycetes* often exhibit enhanced enzymatic stability and activity under variable environmental conditions, making them suitable for diverse biotechnological applications.

Despite their potential, chromogenic *Actinomycetes* from freshwater habitats remain insufficiently explored, particularly with respect to their enzymatic capabilities. Therefore, the present study focuses on exploring the enzymatic potential of chromogenic *Actinomycetes,* aiming to identify novel strains with significant industrial and biotechnological relevance.

2. MATERIALS AND METHOD

Apparatus

- Conical flasks
- Beakers
- Measuring cylinders
- Test tubes
- Petri plates
- Inoculating loops and needles
- Glass slides
- Cover slips
- Sterile sampling bottles

Culture Media

- Starch Casein Agar (SCA)
- Actinomycetes Isolation Agar (AIA)
- Christensen Urea Agar
- Starch Agar
- Skim Milk Agar

Antifungal Agents

- Fluconazole
- Itraconazole

METHOD

The present study deals with screening of industrially important enzymes Amylase, Protease, Oxidase, Catalase and Urease producing aquatic chromogenic *Actinomycetes*.

1. Sample Collection

Water samples were collected from selected aquatic environments of Vidarbha region under aseptic conditions. Samples were collected in sterile containers, labelled properly, and transported to the laboratory. All samples were processed within 24 hours of collection.

2. Isolation of chromogenic *Actinomycetes*

Isolation of Chromogenic *Actinomycetes* was done based on cultural, morphological and biochemical characteristics.

3. Morphological Characterization (Bergey's Manual of Determinative Bacteriology, 1994)

Isolated colonies were examined for Colony Size, shape, Colony colour (aerial and substrate mycelium), Pigment production, Surface texture, Margin and elevation Gram staining was performed, and microscopic examination was carried out to observe filamentous branching mycelia typical of Actinomycetes.

4. Biochemical Characterization

The pure isolates were subjected to standard biochemical tests, viz. Carbohydrate fermentation, IMViC test, Catalase test, Oxidase test, and Urease test.

5. Screening for Enzymatic Potential

Primary screening for extracellular enzyme production was carried out using plate assay methods.

(i) Amylase Production

Isolates were inoculated on starch agar plates. After incubation, grams iodine stain was flood on the plates and left for 5 minutes. After five minutes' clear zones around colonies indicates positive starch hydrolysis. [23]

(ii) Protease Production

Isolates were inoculated on skim milk agar plates. After incubation clear zones around colonies indicates positive protease production. [8]

(iii) Urease Production

The isolates were inoculated on Christensen's Urease Agar medium containing phenol red as a pH indicator. after incubation change in the colour of medium indicates positive urease activity. [22]

(iv) Catalase Production

A small amount of bacterial culture was placed on a clean glass slide and a drop of 3% hydrogen peroxide was added. Immediate bubble formation indicated a positive catalase reaction. [23]

(v) Oxidase Production (Oxidase Disc Method)

A sterile loop was used to smear the culture onto a commercially available oxidase disc. The reaction was observed within 10–30 seconds. The development of a dark purple colour indicated a positive oxidase reaction.

Pure cultures were maintained on *Actinomycetes* isolation agar and starch casein agar slants at 4°C for short-term storage. [23]

3. RESULTS AND DISCUSSION

Table- 1: Collection of Aquatic samples from different locations of Vidarbha region

Sr. No.	Name of Location	Source of Water	Chromogenic *Actinomycetes* observed	Colour of Colony
1	Kaulkhed	Tap Water	+ve	Yellow
2	Choti Umari	Tap Water	+ve	Yellow
3	Khadki	Bore water	+ve	Golden Yellow
4	Washim	Bore water	+ve	Light Yellow
5	Maratha Nagar	Tap Water	+ve	Butter Yellow
6	Malkapur	Tap Water	+ve	Lemon Yellow
7	Chikhaldara	Lake	+ve	Golden Yellow
8	Chikhaldara	Waterfall	+ve	Yellow
9	Chikhaldara	Lake	+ve	Orange
10	Malkapur	Bore water	+ve	Lemon Yellow

11	Malkapur	Bore water	+ve	Cream White
12	Laxmi Nagar	Bore water	+ve	Yellow
13	Akot Road	Bore water	+ve	Orange
14	Vidrupa River	River	+ve	Light Pink
15	Malkapur	Well	+ve	Cream White
16	Morna River	River	+ve	Pitch/Yellow
17	Deshmukh Fail	Hand Pump	+ve	Cream
18	Deshmukh Fail	Tap Water	+ve	Faint Orange

A total of forty aquatic water samples were collected from different locations of vidarbha region including tap water, bore water, lake, river, well, waterfall, and hand pump sources. Forty out of eighteen samples showed positive (+ve) growth for chromogenic *Actinomycetes,* indicating their wide distribution in different aquatic environments.

Table- 2: Frequency distribution of Chromogenic *Actinomycetes* from various aquatic sources of Vidarbha Region

Sr. No.	Source of Water	No. of Samples	No of Isolates	Frequency of Chromogenic *Actinomycetes* in Percentage (%)
1	Tap Water	18	05	28 %
2	Bore water	15	06	33 %
3	River	02	02	11 %
4	Lake	02	02	11 %
5	Waterfall	01	01	5 %
6	Hand pump	01	01	6 %
7	Well	01	01	6 %

Among all sources, bore water showed the highest frequency of chromogenic *Actinomycetes* (6 isolates; 33%), followed by tap water (5 isolates; 28%). River and lake water each yielded 2 isolates (11% each), while waterfall, well and hand-pump water showed the lowest frequency with 1 isolate each (approximately 5–6%).

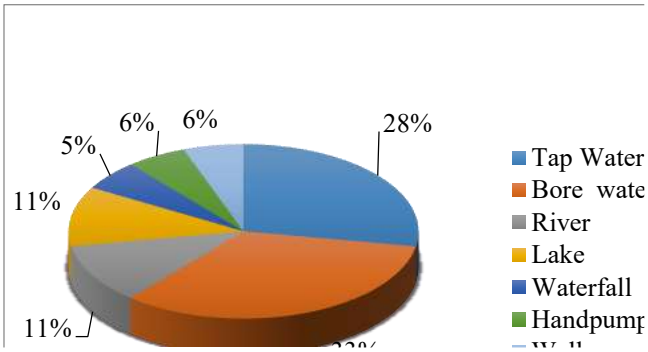

Chart- 1: Frequency distribution of Chromogenic Actinomycetes from various aquatic sources of Vidarbha Region

The results indicate that underground water sources such as bore water and tap water (which is often supplied from underground sources) harbored a higher proportion of chromogenic *Actinomycetes* compared to surface water bodies.

Table- 3: Growth of Chromogenic *Actinomycetes* on selective media

Sr. No.	Name of Media	pH	Temperature	Colour of Colony	Time Period required for pigment Production
1	Starch Casein Agar	Neutral	26 – 38°C	Yellow	6 – 7 days
2	Starch Casein Agar	Neutral	26 – 38°C	Yellow	6 – 7 days
3	Starch Casein Agar	Neutral	26 – 38°C	Golden Yellow	6 – 7 days
4	Starch Casein Agar	Neutral	26 – 38°C	Light Yellow	6 – 7 days
5	Starch Casein Agar	Neutral	26 – 38°C	Butter Yellow	6 – 7 days
6	Starch Casein Agar	Neutral	26 – 38°C	Lemon Yellow	6 – 7 days
7	Starch Casein Agar	Neutral	26 – 38°C	Golden Yellow	6 – 7 days
8	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Yellow	6 – 7 days
9	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Orange	6 – 7 days
10	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Lemon Yellow	6 – 7 days
11	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Cream White	6 – 7 days
12	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Yellow	6 – 7 days
13	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Orange	6 – 7 days
14	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Light Pink	6 – 7 days
15	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Cream White	6 – 7 days

16	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Pitch/Yellow	6 – 7 days
17	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Cream	6 – 7 days
18	*Actinomycetes* Isolation Agar	Neutral	26 – 38°C	Faint Orange	6 – 7 days

The growth pattern of chromogenic *Actinomycetes* on selective media is presented in Table No 3. All eighteen isolates showed good growth on both Starch Casein Agar (SCA) and *Actinomycetes* Isolation Agar (AIA) at neutral pH and temperature ranging from 26–38°C. Pigment production was observed in all isolates within 6–7 days of incubation.

On Starch Casein Agar, isolates predominantly produced yellow colour pigments including yellow, golden yellow, light yellow, butter yellow, and lemon yellow. Golden yellow pigmentation was observed in two isolates, while variations of pale to intense yellow shades were common.

Shirling and Gottlieb (1966), reported that most Chromogenic *Actinomycetes* species exhibit optimal growth under mesophilic conditions with neutral pH and produce characteristic pigments on selective media.

The predominance of yellow and golden-yellow pigments observed on Starch Casein Agar supports the observations of Goodfellow and Williams (1983), described that Chromogenic *Actinomycetes* frequently produce diffusible and non-diffusible pigments ranging from yellow to orange depending on medium composition.

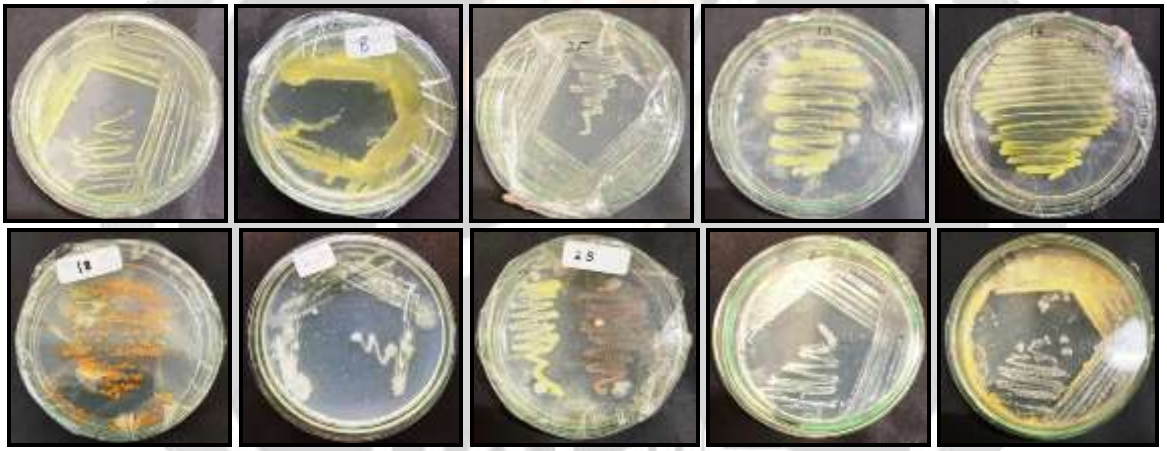

Fig-1: Growth of chromogenic *Actinomycetes* on selective agar with prominent pigmentation

Fig-2: Microscopic view of chromogenic *Actinomycetes*

Table- 4: Cultural and Morphological characteristics of Chromogenic *Actinomycetes*

Isolate No.	Texture	Margin	Elevation	Surface	Colour of Pigment	Gram Nature	Mycelium
A1	Powdery	Irregular	Raised	Smooth	Yellow	+ve	Septate

A3	Slimy	Irregular	Raised	Smooth	Golden Yellow	+ve	Septate
A4	Slimy	Irregular	Raised	Smooth	Light Yellow	+ve	Septate
A5	Slimy	Irregular	Raised	Smooth	Butter Yellow	+ve	Septate
A6	Slimy	Irregular	Raised	Smooth	Lemon Yellow	+ve	Septate
A9	Powdery	Irregular	Raised	Sticky	Orange	+ve	Septate
A16	Velvety	Entire	Raised	Smooth	Pitch/Yellow	+ve	Septate
A18	Velvety	Irregular	Raised	Sticky	Faint Orange	+ve	Septate

The morphological characteristics of eighteen chromogenic *Actinomycetes* isolates are presented in Table No 4. All isolates exhibited distinct colony morphology with noticeable variation in texture, margin, elevation, surface characteristics, and pigment production. Microscopic examination revealed that all isolates were Gram-positive and possessed septate mycelium.

The irregular margins and raised elevation observed in most isolates agrees with the findings of Shirling and Gottlieb (1966), who reported that members of the genus *Streptomyces* typically exhibit raised colonies with irregular margins due to extensive aerial mycelial growth. The presence of septate mycelium in all isolates further supports their classification as filamentous *Actinomycetes*

According to Goodfellow and Williams (1983) Variation in colony texture such as powdery, velvety, chalky, and leathery appearance reflects differences in aerial mycelium and spore chain formation.

Table- 5: Carbohydrate fermentation test of chromogenic *Actinomycetes*.

Isolate No.	Glucose		Lactose		Sucrose		Maltose	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
A1	+ ve	- ve	+ ve	- ve	+ ve	- ve	+ ve	- ve
A3	+ ve	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve
A4	+ ve	- ve	- ve	- ve	- ve	- ve	+ ve	- ve
A5	+ ve	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve
A6	+ ve	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve
A9	+ ve	- ve	+ ve	- ve	- ve	- ve	- ve	- ve
A14	+ ve	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve
A15	+ ve	- ve	- ve	- ve	+ ve	- ve	+ ve	- ve
A16	- ve	- ve	+ ve	- ve	- ve	- ve	+ ve	- ve
A18	+ ve	- ve	- ve	- ve	+ ve	- ve	+ ve	- ve

(+ve: Positive; –ve: Negative)

All 18 isolates were able to ferment glucose with acid production, while gas production was not observed in any isolate. Variable fermentation patterns were observed for lactose, sucrose, and mannitol, indicating metabolic diversity among the *Actinomycetes* isolates. Several isolates such as A5, A11, and A17 showed the ability to ferment multiple sugars, whereas isolates like A4, A10, and A16 fermented only limited sugars.

Biochemical characteristics of Chromogenic *Actinomycetes*

All eighteen isolates (A1–A18) showed negative results for Indole production, Methyl Red test, Voges–Proskauer test, and Citrate utilization. No color change or characteristic reaction was observed in any of the biochemical test performed. In the present study, all chromogenic *Actinomycetes* isolates exhibited negative reactions for Indole, MR, VP, and Citrate utilization tests.

The absence of indole production in all isolates suggests lack of tryptophanase enzyme activity. This finding is consistent with the observations of Shirling and Gottlieb (1966), who reported that most species of *Streptomyces* are indole negative. Similarly, Goodfellow and Williams (1983) described that *Actinomycetes* generally do not possess metabolic pathways associated with enteric bacteria, including tryptophan degradation leading to indole formation.

The negative Methyl Red and Voges–Proskauer results observed in this study indicate that the isolates do not utilize fermentative pathways for glucose metabolism. According to Williams et al. (1989), members of the genus *Streptomyces* are strictly aerobic and depend mainly on oxidative metabolism rather than fermentative pathways. The absence of mixed acid or butylene glycol fermentation in the present isolates therefore supports their aerobic nature.

Similarly, citrate utilization was negative in all isolates. This observation aligns with descriptions in Bergey's Manual of Systematic Bacteriology, which states that many *Actinomycetes* require complex organic substrates and may not utilize citrate as a sole carbon source under standard IMViC test conditions.

Table- 6: Enzyme Potential of chromogenic *Actinomycetes*

Isolate No.	Amylase	Protease	Oxidase	Catalase	Urease
A1	+ve	-ve	-ve	+ve	+ve
A2	+ve	-ve	-ve	+ve	+ve
A3	+ve	+ve	-ve	+ve	+ve
A4	+ve	-ve	-ve	+ve	+ve
A5	+ve	-ve	-ve	+ve	+ve
A6	+ve	+ve	-ve	+ve	+ve
A7	+ve	+ve	-ve	-ve	-ve
A8	+ve	-ve	-ve	+ve	+ve
A9	+ve	-ve	-ve	+ve	+ve

A10	+ve	+ve	-ve	+ve	+ve
A11	+ve	-ve	-ve	+ve	+ve
A12	+ve	+ve	-ve	+ve	+ve
A13	+ve	-ve	-ve	+ve	+ve
A14	+ve	-ve	-ve	+ve	+ve
A15	+ve	-ve	-ve	+ve	+ve
A16	-ve	-ve	-ve	+ve	-ve
A17	+ve	-ve	-ve	+ve	+ve
A18	+ve	-ve	-ve	+ve	+ve

The enzyme production profile of eighteen chromogenic *Actinomycetes* isolates (A1–A18) is presented in Table No 7. The isolates were screened for five extracellular enzymes: Amylase, Protease, Oxidase, Catalase, and Urease.

Amylase activity was observed in seventeen out of eighteen isolates. Only isolate A16 showed negative amylase activity, while all other isolates exhibited positive starch hydrolysis.

Protease activity was seen in only five isolates (A3, A6, A7, A10, and A12), whereas the remaining isolates showed negative results for protease production. Also All eighteen isolates were negative for oxidase activity.

Catalase activity was observed in seventeen isolates. Only isolate A7 was negative for catalase production, while the rest exhibited positive catalase reaction.

Urease activity was detected in the majority of isolates. Isolates A7 and A16 showed negative urease activity, whereas the remaining isolates were urease positive

Overall, the enzyme screening indicates strong amylase and catalase activity among the isolates, moderate urease activity, limited protease production, and absence of oxidase activity.

a. Amylase test b. Protease test c. Oxidase test

d. Catalase test e. Urease test

Fig -3: Enzymatic Activities of chromogenic Actinomycetes

4. CONCLUSION

- Chromogenic *Actinomycetes* is having ability to produce distinct pigments often correlates with the ability to produce the diverse extracellular enzymes such as Amylase, Protease, Oxidase, Catalase and Urease.
- The significant enzymatic potential making them valuable micro-organisms for various industrial applications.
- Enzymes may provide additional benefits such as Antimicrobial or Antioxidant properties.

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