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Studies on Optimization of Growth Parameters for Mass Multiplication of *Actinobacteria*

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Authors' contributions

This work was carried out in collaboration between both the authors. Author BSN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RM managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To isolate, characterize and optimize the growth parameters for mass multiplication of *Actinobacteria*.
Place and Duration of Work: The study was carried out in Department of Agricultural Microbiology, GKVK, University of Agricultural Sciences, Bangalore during 2019-20.
Methodology: Actinobacterial isolates were characterized morphologically and screened for optimization of growth parameters *viz.,* pH, temperature, salt concentration and utilization of carbon source for their mass multiplication.
Results: Forty actinobacterial isolates were enumerated from rhizosphere soil of finger millet, cowpea and also from different organic manures. Color of aerial mycelium in most of the actinobacterial isolates were white, grey or cream with dry, cottony or powdery appearance. All forty isolates were Gram positive, non-acid forming and motile. During optimization of growth parameters, results showed that all the actinobacterial isolates growth was observed good at 30℃, pH 7 and 2 per cent NaCl concentration. Starch was confirmed as the best carbon source for all the actinobacterial isolates during carbon source utilization ability.

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Conclusion: Based on the results, it is showed that all the actinobacterial isolates enumerated were aerobic, spore-forming, Gram positive bacteria, non-acid forming and motile. Maximum growth of *Actinobacterial* isolates was obtained at temperature of 30℃, pH 7 and 2 per cent NaCl concentration with the ability of growing on ten different carbon sources during the optimization of nutritional and cultural characterization studies. Among the different carbon sources, starch was confirmed as the best carbon source for all the isolates during the study of carbon source utilization ability.

Keywords: Actinobacteria; screening; morphology; temperature; pH; NaCl and carbon source.

1. INTRODUCTION

Actinobacteria are a group of prokaryotic microorganisms which are Gram-positive bacteria with high guanine-cytosine in their DNA [1,2]. They are considered as biotechnologically important organisms since they are responsible for producing about half of the bioactive secondary metabolites including antibiotics [3]. They are filamentous bacteria which produce two types of branching mycelium, namely aerial and substrate mycelium. The aerial mycelium are of various colours such as white, grey, red, green, blue and violet series, and the various colours of the substrate mycelia vary from grey to greyish yellow to orange, moderate yellow, pale red greyish orange pin, bluish grey and white [4]. Melanoid pigments are greenish brown, brownish black or distant black. Reverse-side pigments are pale yellow, olive or yellowish brown. Soluble pigments are recorded as red, orange, green, yellow, blue and violet. Spore surface are observed under electron microscope and are characterized as smooth, spiny, hairy and warty [5]. The culture conditions for the isolation include enrichment, selective media such as International *Streptomyces* Project (ISP) media (1-7), starch casein agar, Kuster's agar and asparagine–glucose agar [6]. Factors influencing the number and types of actinobacteria present in a particular soil are geographical location, such as soil type, temperature, organic matter content, moisture content, cultivation and aeration.

Actinobacteria act as a major component of the microbial population in most of the soil types. About 90 per cent of the total *Actinobacteria* population consists of *Streptomyces* species. Soil actinomycetes can produce a wide range of secondary metabolites and about 70 per cent of the naturally derived antibiotics that are currently in clinical use are produced by them. Various traditional biochemical tests [7] and molecular methods like 16s rRNA sequencing followed by BLAST can be done to identify them. They are used as plant growth promoters, biocontrol agents, biopesticides and also as a source of agro-active compounds [8]. The current study was undertaken to isolate, characterize and optimize the nutritional and cultural growth parameters for mass multiplication actinobacteria from different samples.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The soil samples for the isolation of *Actinobacteria* were collected from the rhizosphere of cowpea and finger millet crop fields, University of Agricultural Sciences, Bangalore, in labelled sterile polythene bags and brought to the laboratory and preserved in a refrigerator condition (at 4°C) for further studies along with soil and different organic manure samples were also used for isolation of actinobacterial isolates. Isolation of *Actinobacteria* was done by dilution plate method after serial dilution of soils.

2.2 Characterization of *Actinobacteria*

2.2.1 Colony morphology

Colony characters like shape, colour and pigmentation were recorded by referring to the 9th edition of Bergey's manual of determinative bacteriology (Holt et al., 1994).

2.2.2 Gram staining

Fresh culture of each actinobacterial isolate was placed on a microscopic slide and a smear was prepared. Slides were heat fixed and flooded with crystal violet for one minute, washed with water, then flooded with Gram's iodine for 30 seconds and rinsed with water. Slides were decolorized with alcohol until the solvent flows colourless from the slide, then flooded with safranine for 30 seconds, washed with water, air

dried and examined for Gram reaction under microscope.

2.2.3 Acid fast staining

Smears of actinobacteria were prepared and heat fixed. Slides were placed on the wire gauze on a ring stand and slides were covered with blotting paper and saturated with carbolfuchsin. Slides were heated till steaming but not boiled. Steaming was done for 3-5 minutes continuously by adding few drops of carbolfuchsin to keep the slide moist. At the end of staining, blotting paper was removed and slides were washed with slow jet of water and decolorized with acid-alcohol for 30 seconds. Slides were again washed with water and counter stained with methylene blue for 45 seconds. Finally, slides were washed with water, air dried and examined for acid fastness of the culture.

2.2.4 Cover slip culture technique

The growth of *Actinobacteria* was studied by cover slip culture technique. In this technique, culture plates were prepared using starch casein agar. The coverslip cultures were then taken out from the culture plates and placed on glass slides, with the culture face of the coverslip facing the glass slide. The coverslip-mounted glass slides were then observed under the microscope. Morphological characterization is the first step for characterizing *Actinobacteria* as it helps in preliminary identification of *Actinobacteria.*

2.2.5 Motility test

The motility of actinomycetes was tested by stab culture method. Sulfide-Indole- Motility (SIM) agar medium was used to detect motility. After stabbing the test cultures in SIM agar medium in test tubes, they were incubated at 37^0C for 48 hours. If there is diffused growth of organism in the medium, then the organism is said to be motile or if the growth is confined only to the line of inoculation it is confirmed as non-motile.

2.3 Optimization of Nutritional and Cultural Characterization of Actinobacterial Isolates for Mass Multiplication Parameters

2.3.1 Growth temperature range test [9]

The starch casein agar medium (Soluble starch-10.0 g, Dipotassium hydrogen phosphate (K_2HPO_4) - 2.0 g, Magnesium sulphate ($MgSO_4.7H_2O$)- 0.05 g, Potassium nitrate -2.0 g, Sodium chloride - 2.0 g, Casein - 0.4 g, Ferrous sulfate ($FeSO_4.7H_2O$)- 0.01 g, Calcium carbonate - 0.02 g, Agar -18.0 g, Distilled water - 1000ml, pH -7.2) plates were inoculated with the selected isolate and incubated at 4°C, 25°C, 30°C, 35°C, 40°C, 45°C and 50°C. The extent of growth was recorded on 8^{th} day.

2.3.2 Growth pH range test [9]

The starch casein agar medium slants which were previously adjusted to various pH range starting from pH 5.0, 6.0,7.0, 8.0, 9.0 and 10.0 and they were incubated at 28°C for 7 days and the presence or absence of growth was noted.

2.3.3 Sodium chloride tolerance test [9]

This test was carried out on Bennett`s agar medium (Yeast extract-1.0 g, Beef extract -1.0 g, Casein enzyme hydrolysate -2.0 g, Dextrose-10.0 g, Distilled water-1000 ml, Agar - 15.0 g, pH - 7.3, amended with glycine 4.4 g/ltr). Different concentrations of sodium chloride (2%, 5%, 7% and 10%) solution were added to the starch casein agar medium to check the sodium chloride tolerance test (Kalyani et al*.,* 2012). This test was very much important to understand the native nature of the actinomycetes isolates. The isolate was streaked on the agar medium, incubated at 37°C for 7 days and the presence or absence of growth was noted.

2.3.4 Carbon source utilization ability test

The ability of strains to utilize and produce acid from various carbon sources was studied by the method recommended in the International *Streptomyces* Project. Utilization of carbon sources like starch, mannitol, raffinose, glucose, galactose, sucrose, fructose, maltose and citric acid were tested on basal carbohydrate utilization agar (ISP 9) ($(NH_4)_2SO_4$- 2.64 g, KH_2PO_4- 2.38 g, K_2HPO_4 $3H_2O$- 5.65 g, $MgSO_4$ $7H_2O$- 1.00 g, Distilled water-1000 ml, pH- 6.8 – 7.0, Agar- 15.0 g). Carbohydrate solution (1%) of each carbon source was added to sterile basal media and the media was dispensed into petri plates. The isolates were inoculated into respective carbohydrate agar plates and incubated at 28°C for 5-7 days. the presence or absence of growth was noted.

3. RESULTS AND DISCUSSION

3.1 Colony Morphology of Actinobacterial Isolates

The actinobacterial colonies were found to be distinct by their cottony white, leathery, powdery and mealy appearance on the solid media. The color of aerial mycelium in most of the isolates was white, grey or cream with dry, cottony or powdery appearance which were later identified as *Streptomyces* isolates. It was found that all forty isolates were Gram positive, non-acid fast and motile (Table 1). *Actinobacteria* are aerobic spore forming, Gam-positive bacteria; containing high guanine to cytosine ratio (57-75%) in their genome, and belong to the order Actinomycetales characterized by the presence of both substrate and aerial mycelial growth [10].

3.2 Optimization of Nutritional and Cultural Characterization of Actinobacterial Isolates

The environmental and cultural conditions are critically important for the cell growth [11]. Parameters like initial pH, temperature, etc. have a profound effect on growth of *Actinobacteria*.

3.3 Effect of Temperature on Growth of Actinobacterial Isolates

Normally *Actinobacteria* are sensitive to temperature [12]. The temperature is one of the critical factors that influence the growth of *Actinobacteria*. Maximum growth was obtained at temperature of 30°C. It was noticed that an increase in temperature from 25°C to 35°C increased the growth. However, beyond 35°C, only few isolates have seen for their positive growth (Table 2). In terms of its optimum temperature for growth, the *Actinobacteria* appears to be mesophilic [13]. This observation proved that low temperature may cease the metabolic activity of the actinobacterial isolates and high temperature kills the cell of the *Actinobacteria*.

3.4 Effect of pH on Growth of Actinobacterial Isolates

The effect of pH on growth by the isolated actinobacteria was studied in starch casein medium with different pH. The maximum growth was observed at pH 7. It was noted that with an initial increase in pH (6-8), the growth was also increased, and it decreased significantly above pH 8 (Table 3). Subathra et al., [12] reported that the pH plays an important role in growth of *S. erythraea* and erythromycin production.

3.5 Effect of Sodium Chloride Concentration on Growth of Actinobacterial Isolates

When the actinobacterial isolates were inoculated on starch casein agar media with different NaCl concentrations, it was found that

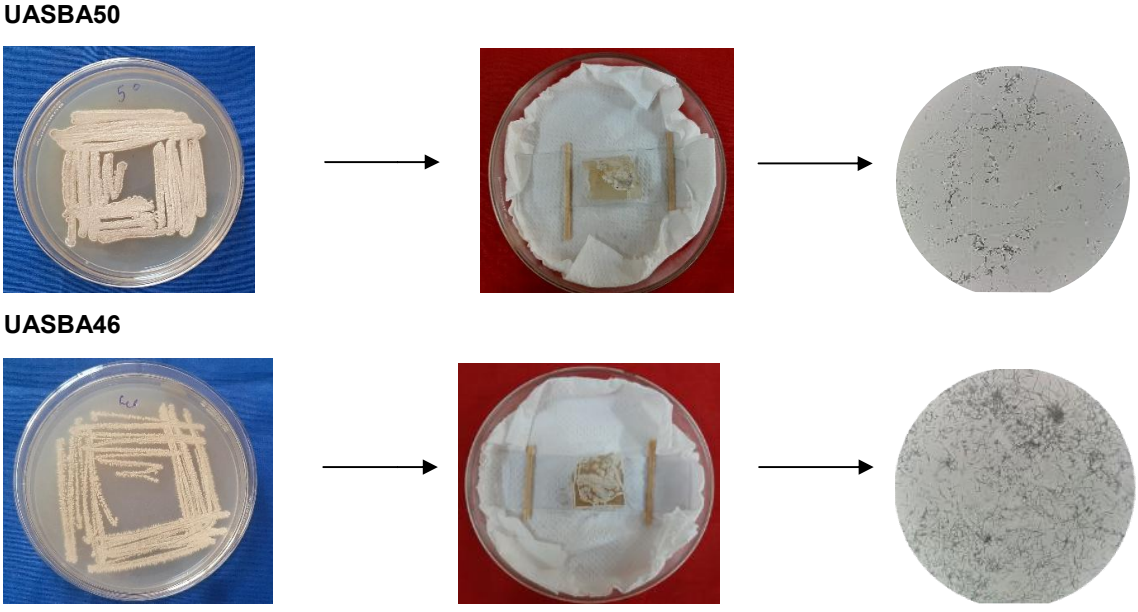

Fig. 1. Cover slip culture technique for efficient actinobacterial isolates

optimum NaCl concentration for the growth of was 2 per cent (Table 4). Some actinobacterial isolates have shown positive growth at 5 per cent sodium chloride concentration. It was observed as the concentration of NaCl in the medium increased, a decrease in the growth was observed. Similar results were observed by Kumar and Kannabiran [14]. *Streptomyces* spp. VITSVK9 showed the maximum growth at 5 per cent of NaCl concentration in the medium. The growth of the strain was inhibited in the absence of NaCl in the medium. Carbon, nitrogen, amino acid sources, culturing conditions, incubation temperature, pH and time period have profound influence on the growth by *Streptomyces* VITSVK9 spp.

Table 1. Morphological characteristics of actinobacterial isolates

Sl. No.	Actinobacterial isolates	Gram reaction	Motility	Acid fast	Morphological features	
					Colour	Shape
1.	UASBA1	+ve	+	-	White	Irregular
2.	UASBA2	+ve	+	-	Grey	Irregular
3.	UASBA3	+ve	+	-	White	Circular
4.	UASBA5	+ve	+	-	White	Circular
5.	UASBA6	+ve	+	-	White	Filamentous
6.	UASBA7	+ve	+	-	Cream	Circular
7.	UASBA12	+ve	+	-	Orange	Circular
8.	UASBA13	+ve	+	-	Yellow	Irregular
9.	UASBA15	+ve	+	-	White	Irregular
10.	UASBA18	+ve	+	-	White	Irregular
11.	UASBA21	+ve	+	-	Cream	Irregular
12.	UASBA22	+ve	+	-	White	Irregular
13.	UASBA24	+ve	+	-	White	Filamentous
14.	UASBA25	+ve	+	-	Red	Circular
15.	UASBA26	+ve	+	-	Yellow	Irregular
16.	UASBA27	+ve	+	-	Red	Circular
17.	UASBA28	+ve	+	-	Red	Circular
18.	UASBA30	+ve	+	-	White	Circular
19.	UASBA31	+ve	+	-	White	Irregular
20.	UASBA32	+ve	+	-	Cream	Circular
21.	UASBA34	+ve	+	-	White	Irregular
22.	UASBA35	+ve	+	-	White	Irregular
23.	UASBA36	+ve	+	-	White	Rhizoid
24.	UASBA39	+ve	+	-	Yellow	Irregular
25.	UASBA46	+ve	+	-	White	Filamentous
26.	UASBA47	+ve	+	-	Grey	Filamentous
27.	UASBA49	+ve	+	-	Grey	Circular
28.	UASBA50	+ve	+	-	Grey	Circular
29.	UASBA54	+ve	+	-	Grey	Circular
30.	UASBA60	+ve	+	-	White	Circular
31.	UASBA62	+ve	+	-	Cream	Filamentous
32.	UASBA63	+ve	+	-	Grey	Filamentous
33.	UASBA65	+ve	+	-	White	Circular
34.	UASBA66	+ve	+	-	Yellow	Circular
35.	UASBA67	+ve	+	-	Grey	Circular
36.	UASBA72	+ve	+	-	White	Filamentous
37.	UASBA74	+ve	+	-	White	Circular
38.	UASBA75	+ve	+	-	Orange	Circular
39.	UASBA76	+ve	+	-	Red	Circular
40.	UASBA77	+ve	+	-	Orange	Circular

Note: +: Positive, -: Negative

Table 2. Effect of temperature on growth of actinobacterial isolates

Sl. No.	Actinobacterial isolates	Temperature (°C)						
		4	25	30	35	40	45	50
1.	UASBA1	-	+	+	-	-	-	-
2.	UASBA2	-	+	++	+	-	-	-
3.	UASBA3	-	++	+++	++	+	-	-
4.	UASBA5	-	+++	+	++	++	-	-
5.	UASBA6	-	+	+	+	-	-	-
6.	UASBA7	-	+	++	+	-	-	-
7.	UASBA12	-	+	++	+	-	-	-
8.	UASBA13	-	++	++	+	-	-	-
9.	UASBA15	-	+	+	-	-	-	-
10.	UASBA18	-	++	++	++	-	++	-
11.	UASBA21	-	+	+	-	-	-	-
12.	UASBA22	-	++	++	++	++	-	-
13.	UASBA24	-	+++	++	++	++	+	-
14.	UASBA25	-	+	+	-	-	-	-
15.	UASBA26	-	+++	+++	+++	++	-	-
16.	UASBA27	-	+++	+++	+++	++	+	-
17.	UASBA28	-	++	+++	+++	++	-	-
18.	UASBA30	-	+	+	-	-	-	-
19.	UASBA31	-	+	+	-	-	-	-
20.	UASBA32	-	+++	+++	+++	++	+	-
21.	UASBA34	-	+	+	-	-	-	-
22.	UASBA35	-	+	+	-	-	-	-
23.	UASBA36	-	+	+	+	-	-	-
24.	UASBA39	-	+	++	+	+	-	-
25.	UASBA46	-	+++	+++	+++	++	+	-
26.	UASBA47	-	+	+	-	-	-	-
27.	UASBA49	-	+	+	-	-	-	-
28.	UASBA50	-	+++	+++	+++	++	+	-
29.	UASBA54	-	+	+	+	-	-	-
30.	UASBA60	-	++	+++	+++	+	+	-
31.	UASBA62	-	++	++	++	+	-	-
32.	UASBA63	-	+	+	-	-	-	-
33.	UASBA65	-	+	+	-	-	-	-
34.	UASBA66	-	+	+	-	-	-	-
35.	UASBA67	-	++	++	++	++	++	-
36.	UASBA72	-	+	+	-	-	-	-
37.	UASBA74	-	+	+	+	+	-	-
38.	UASBA75	-	+	+	-	-	-	-
39.	UASBA76	-	+	+	-	-	-	-
40.	UASBA77	-	+	+	-	-	-	-

Note: -: No growth, +: Poor growth, ++: Medium growth, +++: Excellent growth

Isolation and characterization of antibiotic-producing actinobacteria were studied by Kalyani et al. [9] in which they also studied physiological properties of the isolates. They studied growth of actinobacterial isolates at different temperatures (4°C, 20°C, 30°C, 42°C and 50°C) at different pH (5.8, 7, 9) and NaCl tolerance (2%, 5% and 7%) and found that the all three isolates showed growth at all the tested temperatures except 40°C and at pH 7 and 9. Salt concentration at 2.5 per cent was tolerated by all the isolates while one isolate showed growth at all the tested salt concentrations.

Table 3. Effect of pH on growth of actinobacterial isolates

Sl. No.	Actinobacterial isolates	pH					
		5.0	6.0	7.0	8.0	9.0	10.0
41.	UASBA1	-	+	+	-	-	-
42.	UASBA2	-	++	++	-	-	-
43.	UASBA3	-	+++	+++	++	+	+
44.	UASBA5	-	+++	+++	+++	++	++
45.	UASBA6	-	+	+	-	-	-
46.	UASBA7	-	+	+	-	-	-
47.	UASBA12	-	+	+	-	-	-
48.	UASBA13	-	+	+	-	-	-
49.	UASBA15	-	+	+	-	-	-
50.	UASBA18	-	+++	+++	+++	++	++
51.	UASBA21	-	+	-	-	-	-
52.	UASBA22	-	+	-	-	++	-
53.	UASBA24	-	++	++	++	+	-
54.	UASBA25	-	+	+	-	-	-
55.	UASBA26	-	++	+++	+++	-	-
56.	UASBA27	-	++	++	++	++	-
57.	UASBA28	-	++	++	++	++	++
58.	UASBA30	-	+	+	-	-	-
59.	UASBA31	-	+	+	-	-	-
60.	UASBA32	-	+++	+++	+++	++	++
61.	UASBA34	-	+	-	-	-	-
62.	UASBA35	-	+	-	-	-	-
63.	UASBA36	-	+	+	-	-	-
64.	UASBA39	-	++	++	++	++	++
65.	UASBA46	-	+++	+++	+++	+++	++
66.	UASBA47	-	+	+	-	-	-
67.	UASBA49	-	+	-	-	-	-
68.	UASBA50	-	+++	+++	+++	++	++
69.	UASBA54	-	+	+	-	-	-
70.	UASBA60	-	++	++	++	++	++
71.	UASBA62	-	+	+	++	+	+
72.	UASBA63	-	+	+	-	-	-
73.	UASBA65	-	+	+	-	-	-
74.	UASBA66	-	+	+	-	-	-
75.	UASBA67	-	++	++	++	++	++
76.	UASBA72	-	+	-	-	-	-
77.	UASBA74	-	+	+	+	-	-
78.	UASBA75	-	+	+	-	-	-
79.	UASBA76	-	+	+	-	-	-
80.	UASBA77	-	+	-	-	-	-

Note: -: No growth, +: Poor growth, ++: Medium growth, +++: Excellent growth

3.6 Carbon Source Utilization Ability by Actinobacterial Isolates

The nutritional sources of carbon are known to have a profound effect on the actinobacterial growth [15]. In the present study, different carbon sources used monosaccharides (fructose, galactose, glucose, mannose), disaccharides (maltose, sucrose) trisaccharides (raffinose) and polysaccharides (starch) were supplemented into starch casein agar medium to determine their impact on growth. The twenty-five actinobacterial isolates were able to grow in all the tested carbon sources but starch was found to be the

best carbon source (Table 5). Ripa et al. [16] found that supplementation of medium with glucose (2%) as sole carbon source produced high levels of antimicrobial metabolites by new *Streptomyces* species (RUPA-08PR) isolated from soil collected from Bangladesh. Jonsbu et al. [17] reported that due to species specific variation, different *Streptomyces* species require different types of carbon sources for cell growth and production of secondary metabolite. Carbon utilization potential of *Actinobacteria* was studied by Lavanyalatha et al. [18] and Thirumalairaj et al. [19] and their results support our observations in the present study.

Table 4. Effect of sodium chloride concentration on growth of actinobacterial isolates

Sl. No.	Actinobacterial Isolates	Sodium chloride concentration (%)			
		2	5	7	10
1.	UASBA1	+	-	-	-
2.	UASBA2	+	-	-	-
3.	UASBA3	++	+	-	-
4.	UASBA5	++	+	-	-
5.	UASBA6	+	-	-	-
6.	UASBA7	+	-	-	-
7.	UASBA12	+	+	-	-
8.	UASBA13	+	-	-	-
9.	UASBA15	+	-	-	-
10.	UASBA18	++	-	-	-
11.	UASBA21	-	-	-	-
12.	UASBA22	++	+	+	-
13.	UASBA24	-	-	-	-
14.	UASBA25	+	-	-	-
15.	UASBA26	++	+	+	-
16.	UASBA27	+	-	-	-
17.	UASBA28	+	-	-	-
18.	UASBA30	+	-	-	-
19.	UASBA31	+	-	-	-
20.	UASBA32	++	+	-	-
21.	UASBA34	+	-	-	-
22.	UASBA35	+	-	-	-
23.	UASBA36	+	-	-	-
24.	UASBA39	+	-	-	-
25.	UASBA46	++	+	+	-
26.	UASBA47	+	-	-	-
27.	UASBA49	+	-	-	-
28.	UASBA50	++	+	-	-
29.	UASBA54	+	-	-	-
30.	UASBA60	++	+	+	-
31.	UASBA62	++	+	+	-
32.	UASBA63	+	-	-	-
33.	UASBA65	+	-	-	-
34.	UASBA66	+	-	-	-
35.	UASBA67	++	-	-	-
36.	UASBA72	+	-	-	-
37.	UASBA74	+	-	-	-
38.	UASBA75	+	-	-	-
39.	UASBA76	+	-	-	-
40.	UASBA77	+	-	-	-

Note: -: No growth, +: Medium growth, ++: Excellent growth

Table 5. Carbon source utilization ability by actinobacterial isolates

Sl. No.	Actinobacterial isolates	Carbon source								
		Glucose	Starch	Raffinose	Glucose	Galactose	Sucrose	Fructose	Maltose	Citric acid
1	UASBA1	+	++	+	+	+	+	+	+	+
2	UASBA3	+	++	+	+	+	+	+	+	+
3	UASBA5	+	++	+	+	+	+	+	+	+
4	UASBA7	+	++	+	+	+	+	+	+	+
5	UASBA12	+	++	+	+	+	+	+	+	+
6	UASBA13	+	++	+	+	+	+	+	+	+
7	UASBA18	+	++	+	+	+	+	+	+	+
8	UASBA21	+	++	+	+	+	+	+	+	+
9	UASBA22	+	++	+	+	+	+	+	+	+
10	UASBA24	+	++	+	+	+	+	+	+	+
11	UASBA26	+	++	+	+	+	+	+	+	+
12	UASBA27	+	++	+	+	+	+	+	+	+
13	UASBA28	+	++	+	+	+	+	+	+	+
14	UASBA30	+	++	+	+	+	+	+	+	+
15	UASBA31	+	++	+	+	+	+	+	+	+
16	UASBA32	+	++	+	+	+	+	+	+	+
17	UASBA34	+	++	+	+	+	+	+	+	+
18	UASBA35	+	++	+	+	+	+	+	+	+
19	UASBA36	+	++	+	+	+	+	+	+	+
20	UASBA39	+	++	+	+	+	+	+	+	+
21	UASBA46	+	++	+	+	+	+	+	+	+
22	UASBA47	+	++	+	+	+	+	+	+	+
23	UASBA50	+	++	+	+	+	+	+	+	+
24	UASBA60	+	++	+	+	+	+	+	+	+
25	UASBA62	+	++	+	+	+	+	+	+	+

Note: -: No growth, +: Medium growth, ++: Excellent growth

4. CONCLUSION

Findings of the study indicated that all the actinobacterial isolates were aerobic, spore-forming Gram positive, non-acid forming, motile bacteria. Among the forty actinobacterial isolates, highest growth was attained at 30°C, pH 7 and 2 per cent NaCl concentration. Starch was confirmed as the best carbon source for all the isolates during the study of carbon source utilization ability.

Further studies on these are underway with respect to the efficiency in the production of different enzymes and screening for their ability on functional and biocontrol traits for effective utilization of the efficient actinobacterial isolate for the sustainable crop production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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