VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

ISOLATION AND CHARACTERIZATION OF CELLULASE – PRODUCING ACTINOMYCETES FROM AGRICULTURAL SOILS OF GUJARAT, INDIA: POTENTIAL FOR LIQUID LIGNOCELLULOSIC WASTE DEGRADATION

Aanal Patani[1], Jaimin Pandya[1], Apoorva Vaghela[1], Jahanvi Thakor[1], Vikram Raval[1], Kiransinh Rajput[1], Rakeshkumar Panchal[1]

[1]Department of Microbiology & Biotechnology, University School of Sciences, Gujarat University, Ahmedabad 380009, Gujarat, India.
Aanal Patani (ORCID: 0000-0003-2399-1110)
Jaimin Pandya (ORCID: 0000-0002-5821-762X)
Apoorva Vaghela (ORCID: 0009-0003-5933-7839)
Jahanvi Thakor (ORCID: 0009-0006-4010-7189)
Vikram Raval (ORCID: 0000-0001-8912-1850)
Kiransinh Rajput (ORCID: 0000-0001-8669-8347)
Rakeshkumar Panchal (ORCID: 0000-0001-8715-8553)

Details of correspondence
Dr. Rakeshkumar R. Panchal
Professor, Department of Microbiology & Biotechnology,
University School of Sciences, Gujarat University,
Ahmedabad 380009, Gujarat, India.
Email: rrpanchal@gujaratuniversity.ac.in

Author's contributions
A.P. and R.P. contribute to the conception and design of the research plan and manuscript. A.P. and J.P. contributed to research execution, compiled the literature, and wrote the manuscript; A.V. and J.T. assisted in data analysis and prepared tables. V.R., K.R., and R.P. reviewed the manuscript.

Abstract

Actinomycetes are known for their ability to produce various extracellular enzymes, including cellulases, which are vital for the degradation of agricultural waste. However, their cellulase production potential has not been extensively studied in soils from Gujarat, India. This study aims to isolate and characterize cellulase-producing actinomycetes from agricultural soils in Gujarat, India, to assess their enzyme production potential. A total of 97 actinomycete strains were isolated from different regions, and their cellulase activity was evaluated using enzyme assays. Total 89 showed significant cellulase activity. Molecular identification using 16S rRNA gene sequencing revealed *Pseudonocardia carboxydivorans* AA4, *Streptomyces griseorubens* AA62, and *Streptomyces violaceorectus* AA74 as the most efficient cellulase producers, with maximum activity reported at 3.22 ± 0.03 U/mL, 3.14 ± 0.02 and 3.06 ± 0.02 U/mL respectively. The cellulase-producing actinomycetes identified in this study could serve as efficient biological agents for the degradation of lignocellulosic waste, with potential applications in sustainable waste management and bioresource utilization.

Keywords: Gujarat; Actinomycetes; Cellulases; *Streptomyces griseorubens* AA62

1.0 INTRODUCTION

With its complex composition of organic matter, inorganic particles, minerals, and microorganisms, soil is the most intricate biomaterial in the ecosystem. For the metabolic processes of microorganisms, it is thought to be a typical medium [7]. It also plays an important role in the Earth's global biogeochemical cycles, which affect the environment and other organisms [8]. Majority of the organic matter in soil is derived from plants. Plant tissues

VIDYA - A JOURNAL OF GUJARAT UNIVERSITY
Peer-Reviewed, Multidisciplinary & Multilingual Journal
ISSN: 2321-1520 **E-ISSN:** 2583-3537

consist of 60 ~ 90 % moisture. At the same time, the remaining dry matter is mostly constituted of carbon (C), oxygen (O2), and hydrogen (H), with trace amounts of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). Despite the minimal value, these nutrients are important for microorganisms [9]. Numerous types of microorganisms, including bacteria, actinomycetes, mold, and archaea, are found in the soil. These organisms participate in the breakdown of organic matter in the soil, and the population dynamics of soil microorganisms, together with the types of organic matter sources, affect the pace of decomposition [6]. These organisms take part in the decomposition of soil organic materials. The diversity of microorganisms in the soil enhances its capacity to decompose a broad spectrum of organic compounds. Gujarat is endowed with a variety of macro- and micro-climates, physiography, landforms, geology, and vegetation, all of which have an impact on the genesis of soil. Over many millions of years, soil systems have been developing. Gujarat has higher levels of nitrogen and potassium in its soil than the rest of India [2].

Actinomycetes of different genera have been studied for the generation of various potential industrial enzymes that may be employed in biotechnological and biomedical fields. This diversity of microorganisms is found in soil. The Greek terms "atkis" (ray) and "mykes" (fungus) are the source of the word "actinomycetes". It describes a class of organisms that have characteristics of fungus and bacteria but vary sufficiently to belong in the kingdom of bacteria. The gram-positive, aerobic actinomycetes have high genomic guanine-cytosine content (57–75%) and are spore-forming bacteria. Like fungi, they are filamentous and feature real aerial hyphae. They are mainly found in dry, alkaline soil in various environments, particularly soil. Actinomycetes are found everywhere and have a steady population. They exhibit a range of unique life cycles compared to other prokaryotes and seem to be essential for the biogeochemical cycle within the soil environment [10][11]. It is also widely recognized that actinomycetes generate a broad variety of secondary metabolites, including immunosuppressants, antifungals, antivirals, antiprotozoals, antibiotics, and antihelminth. They are essential to agriculture as well as the pharmaceutical sectors. Both aerobic and anaerobic, with temperatures ranging from 5-7°C to 45-70°C, actinomycetes can be found in a wide variety. Screening for microbial species is an essential component, as there is a remarkable source for the synthesis of structurally varied primary and secondary metabolites that contain industrially relevant biological activities [10] [11].

Enzymes produced by actinomycetes are amylases, cellulases, catalase, chitinase, lipase, protease, urease, etc. Many genera in the order Actinomycetales have species that actively break down cellulose; these species include both facultatively thermophilic and mesophilic species. The enzymatic degradation of cellulose is a process that involves the combined action of three types of enzymes [12]. Endo-1,4-glucanases initiate the process by randomly rupturing internal links inside the cellulose chain. A series of successive cuts in a single polysaccharide chain that go through the active site come next. A further factor is the enzymatic activity of exo-1,4-glucanases on the cellulose polymer's reducing or non-reducing end. Glucosidases, which are a kind of enzyme, are present in large quantities in both natural and commercial cellulase mixtures. They have been extensively studied. Glucosidases are responsible for converting cellobiose, the primary byproduct of the combination of endo- and exo-glucanases, into glucose [1] [13].

Actinomycetes are constantly evaluated for novel bioactive chemicals and have also produced several significant bioactive molecules with great economic potential. They constitute a formidable group of industrially important microorganisms that have been explored to produce Antibiotics [5]. This Research is continuously focused on the discovery of novel enzymes or the advancement of existing enzymes and secondary metabolites by isolation and screening for the demand of biocatalysts that are robust and have a high turnover rate and are easily and affordably available. Here, the research also intended to determine the potential of actinomycetes to produce antibiotics by isolating and characterizing them from agricultural soil samples.

2.0 MATERIAL AND METHODOLOGY

2.1 Study site

The seven sub-agroclimatic zones of Gujarat, India, were among the locations from which various samples were taken. The built-in GPS app on a mobile phone was used to log the coordinates of each place. The location was chosen based on a multitude of characteristics, including a hot, dry environment; a fertile area; sandy soil; and a place for a location for disposing of waste. 100 g of the soil sample was collected from each site at a depth of ~15 cm, placed in sterile polythene zip lock bags, and delivered to the lab while being kept cold for examination.

2.2 Physicochemical analysis of Soil

According to the laboratory guide for soil analysis, the soil samples were analyzed for many physicochemical parameters, including soil type, soil pH, electrical conductivity (EC), organic carbon, and readily available nitrogen, phosphorus, potassium (K), and sulphur (S).

2.3 Isolation, cultural and morphological characteristics of Actinomycetes

Heat treatment was given to each soil samples in a water bath at 60 °C for 10 min for elimination of non-sporulating bacteria. The spread plate method was used to inoculate 100 µL of each serial dilution onto glucose asparagine agar, starch casein agar, actinomycetes agar medium, and soil extract agar medium. All samples were

ISSN: 2321-1520 **E-ISSN:** 2583-3537

serially diluted in the range of 10^{-1} to 10^{-4}. The plates were incubated for 7–10 days at 28 °C. Colony morphology observations, including measurements of size, shape, margin, pigmentation, configuration, and elevation, were recorded [27]. Morphology of cells were studied by performing Gram's staining and slides were observed under Microscope (Lawrence & Mayo LM – 52 – 3002 Fluorescence Microscope). Further arrangement of cells and spores was studied under scanning electron microscope [3] [12] [14].

2.4 Production of cellulase

2.4.1 Preliminary screening of actinomycetes isolates for cellulase

Actinomycetes strains that differed in terms of colony and morphology were examined using the spot inoculation method and CMC agar medium (carboxy methyl cellulose - 10 g, L-asparagine - 0.5 g, K_2HPO_4 - 0.5 g, Agar - 20 g, Distilled water 1000 mL and pH 7 ± 0.2) for the initial screening of actinomycetes for cellulose degradation. Following two to three days of incubation at 28 °C, the plates were counter stained with 1.0 N sodium chloride solution and stained with 0.1% (w/v) Congo red. By determining the cellulolytic index (CI), which is the ratio of; the zone of hydrolysis diameter to the colony diameter, the cellulolytic potential of the positive isolates was assessed [15] [17].

2.4.2 Secondary screening of actinomycetes isolates for cellulase

To determine the enzyme yield DNSA method was performed from the clear supernatant broth collected aseptically. For that, each chosen strain's spore suspension (10 mL of 1.0 x 10^6 spores/mL) was inoculated in duplicate into a 250 mL Erlenmeyer flask containing 100 mL of CMC broth medium (10 g of carboxymethyl methylcellulose, 0.5 g of L-asparagine, 0.5 g of K_2HPO_4, 1000 mL of distilled water, and pH 7 ± 0.2). In a shaker incubator running at 120 rpm for seven days, flasks were incubated at 28 °C. After 24 hours of fermentation, 2 mL of the samples were removed from each flask and centrifuged for 10 minutes at 4 °C at 10,000 rpm. to determine the enzyme yield, the clear supernatant broth was aseptically collected [16].

2.5 Biochemical and Physiochemical characterization

Colony morphology and arrangements of all three strains were studied on different ISP medium. Utilization of carbon source, utilization of inorganic and organic protein source and production of extracellular enzymes were analyzed by standard protocols. A zone of starch hydrolysis surrounding the actinomycete colonies, which were cultivated on 2% starch agar media for seven days, was seen when 0.1 N iodine was flooded over them. The isolates were grown on 1% tributyrin agar to measure lipase activity, and the presence or absence of a zone surrounding the colony was also noted. Utilization of carbon sources was assessed using nutrient broth containing different sugars, the color change observed by 0.1% Andrade indicator present in media. Citrate utilization was determined the distinct color change on Simmons's citrate agar medium. The development of oxygen bubbles in a 2% hydrogen peroxide solution, the creation of violet color on a paper disc, and the disappearance of 1% methylene blue in nutritional broth medium were used to measure the activities of catalase, oxidase, and dehydrogenase respectively. These tests were studied for biochemical characterization [11] [23].

2.6 Molecular characterization

Chromosomal DNA was extracted by using spin column kit (HiMedia, India or similar manufacturers. Bacterial 16S rRNA gene (1500 bp) [24] was amplified using polymerase chain reaction in a thermal cycler and were purified using Exonuclease I - Shrimp Alkaline Phosphatase (Exo-SAP) [25]. Purified amplicons were sequenced by Sanger method in ABI 3500xL genetic analyzer (Life Technologies, USA) (outsourcing from CSIR-NCIM, Pune, India). Sequence files (.ab1) edited using BioEdit (version 7.2) and further analyzed by Basic Local Alignment Search Tool (BLAST) with closest culture sequence retrieved from the National Centre for Biotechnology Information (NCBI) database that finds regions of local similarity between sequences [26]. MEGA version 11.0 was used for sequence alignment phylogenetic analysis and molecular evolutionary research. By employing UPGMA and the neighbor-joining method, a phylogenetic tree was created [4] [26] [28].

2.7 Application of *S. griseorubens* AA62 in degradation of liquid cellulosic waste

Laboratory-scale treatment was employed for the treatment of liquid textile industrial waste. The collected effluent was initially analyzed for key parameters including pH, temperature, transparency, cellulose content, biological oxygen demand (BOD), chemical oxygen demand (COD), viscosity, and pigmentation. Where pH was measured by pH meter, thermometer was used for the temperature measurement, transparency was measured visually, cellulose content was measured by Sweitzer method and viscosity was measured using Brookfield viscometer DV-I prime. For the treatment process, 250.0 mL of liquid waste was transferred into a sterile 500.0 mL flask. A 13.0% (v/v) inoculum (48 hours grown) of the *S. griseorubens* AA62 was added, and the setup was incubated on a rotary shaker at 150 rpm at room temperature 28 °C for 96 hours.

For the treatment using cell free enzyme, the same process was used where 250.0 mL of liquid waste was transferred into a sterile 500.0 mL flask. 25.0 mL cell free crude enzyme was added, and the setup was incubated on a rotary shaker at 150 rpm at room temperature 28 °C for 96 hours.

Following both the treatments, after completion of 96 hours samples were collected for the analysis to assess the degradation efficiency and changes in effluent properties.

VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

RESULT AND DISCUSSION

3.1 Physicochemical analysis of Soil

The different zone of Gujarat, India, is characterized by sandy to loam soils with sand dunes and arid area [21] [22]. Significant differences seen across soil samples based on their physicochemical properties (Table 1). The temperature varied from 25 to 45 °C, and the pH of the soil was found to be between 8.00 and 9.00. The electric conductivity ranges from 0.34 to 0.73. dSm^{-1}. Soil from North-west arid region is more alkaline with compared to Southern hill region. Total organic carbon, available phosphorous, available potassium and sulphur contents in the soil samples are presented in Table 1. Were total organic carbon ranging from 0.36 to 0.59 (% w/w), available phosphorous found between 10.82 to 48.28 (% w/w), available potassium ranges from 62.50 to 114.2 (µg/g) which shows high potassium content. Sulphur content found in the range of 24.68 to 54.32 (% w/w). Soil samples of Southern Gujarat contain more organic carbon while North Saurashtra contains lowest. Further maximum Potassium available in North Gujarat region. Maximum actinomycetes diversity observed from southern hills region while lowest found in North Gujarat.

3.2 Isolation, cultural and morphological characteristics of Actinomycetes

The characteristics of the colonies were used to describe 97 distinct morphotypes (Figure 1). A total of 15, 12, 13, 03, 22, 18, and 14 distinct colonies were isolated from samples 1, 2, 3, 4, 5, 6, and 7. Colonies are differentiated based on their size, shape, spore arrangement, color of spores and mycelium, color diffusion in media and consistency of colonies. From the various samples a maximum of 28 distinct colonies were isolated on AIA medium; 24 diverse colonies were isolated on soil extract agar media; 19 colonies were found on starch casein agar media; and 26 colonies were isolated on glucose asparagine agar media. Every isolate grew best at 28–30 °C and favored an alkaline pH of 8.0 ± 0.5. Despite this, the actinomycetes were primarily alkaliphilic. Furthermore, according to Table 2, only a few isolates produced pigmented colonies in the colors dark brown, orange, grey, purple, and black. The isolates showed a range of cell shape, size, and colony pattern on different media, and were Gram-positive (table 2). Further colonies are examined using a 10× objective lens, and the filament morphology is noted (Table 2). As shown in table 2 all colonies have different type of filaments.Table 1 Physicochemical characteristics of soil

Sample No.	Sampling Station	Latitude (°N)	Longitude (°E)	Soil Type	Soil pH	E.C. dS m^{-1}	Organic C (% w/w)	Available P (% w/w)	Available K (µg/g)	Available S (% w/w)	No. of actinomycetes obtained	Average CFU/mL ($\times 10^4$)
1.	Middle Gujarat Patan, Gujarat	24.001412	72.093968	Loamy	8.6	0.41	0.48	48.28	94.12	28.62	15	5.6
2.	North-West Arid, Kharaghoda, Gujarat	23.175028	71.696578	Sandy	8.9	0.37	0.39	19.72	72.30	30.23	12	3.4
3.	South Saurashtra Junagadh, Gujarat	21.635050	71.029244	Loamy	8.3	0.73	0.56	27.88	89.52	54.32	13	4.1
4.	North Gujarat Idar, Gujarat	23.874443	73.047121	Sandy	8.2	0.44	0.42	15.64	114.2	45.62	03	0.7
5.	Southern Hills (Dangs - Valsad)	20.598893	73.083209	Loamy	8.1	0.39	0.54	22.43	98.54	38.85	22	8.2

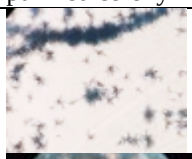
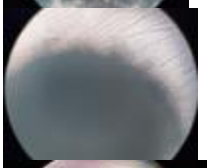
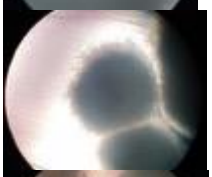
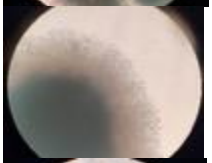
VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

6.	Southern Gujarat Bhadaran, Gujarat	22.370244	72.875970	Peaty	8.4	0.46	0.59	10.82	85.30	42.12	18	6.1
7.	North Saurashtra Dasada, Gujarat	23.353264	71.753305	Sandy	8.8	0.34	0.36	25.84	62.50	24.68	14	4.3

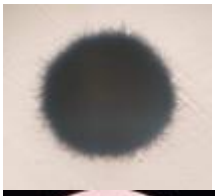
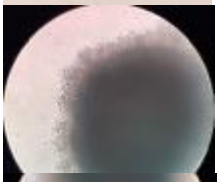
Table 2 Morphological, cultural, and physicochemical characteristics of isolated Actinomycetes

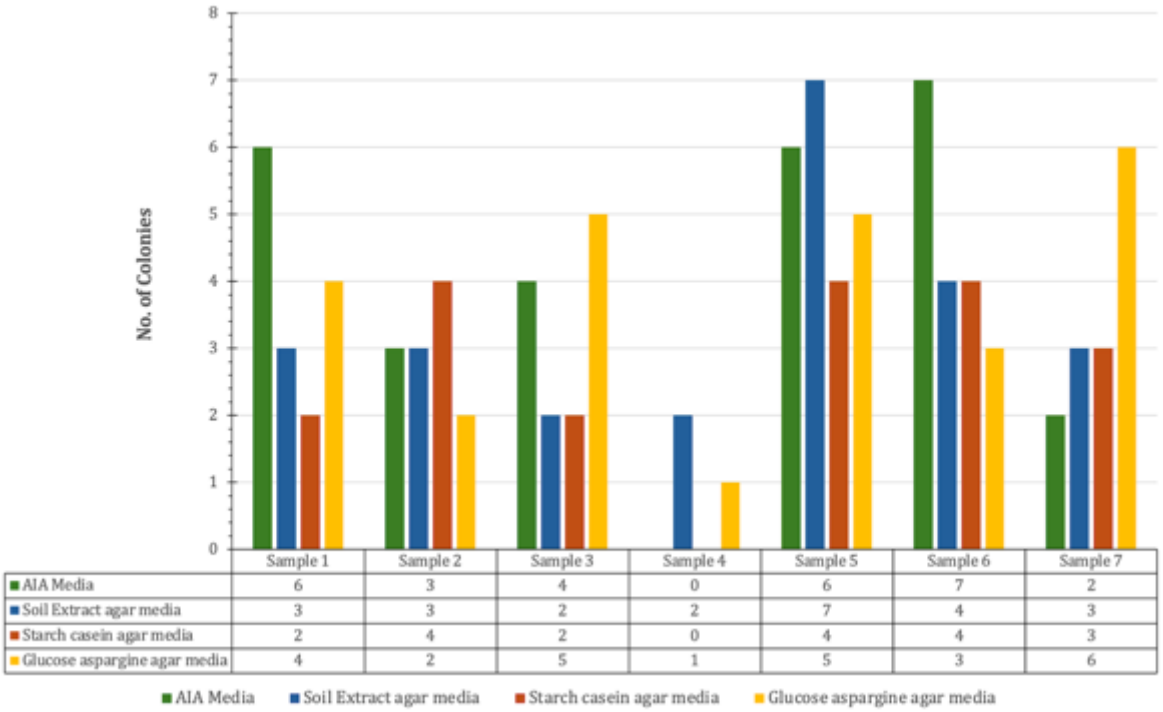
Isolate	Microscopic observation of purified colony	Morphological, cultural, and physicochemical characteristics of Actinomycetes on AIA medium Description (Colony morphology, microscopic features, growth characters)
AA 4		Small, Round, Rectus, Arial mycelium White – Spores White (color diffused in media – dark brown), Dry, Gram's Positive Temp. range 4 to 55 °C (opt. 28 °C), pH range 3 – 11 (opt. 8), rpm range 60 – 200 (opt. 120), salt tolerance up to 6.0 %
AA 19		Small, Uneven, Spira, Arial mycelium White – Spores grey, Dry, Gram's Positive Temp. range 4 to 55 °C (opt. 28 °C), pH range 5 – 11 (opt. 8), rpm range 60 – 200 (opt. 120), salt tolerance up to 8.0 %
AA 24		Medium, Uneven, Spira, Arial mycelium Grey – Spores Grey (color diffused in media), Dry, Gram's Positive Temp. range 4 to 55 °C (opt. 28 °C), pH range 5 – 9 (opt. 8), rpm range 60 – 200 (opt. 120), salt tolerance up to 0.5 %
AA 26		Medium, Round, Monoverticillus-spira, Arial mycelium grey – Spores black, Dry, Flat, Gram's Positive Temp. range 28 to 55 °C (opt. 28 °C), pH range 3 – 9 (opt. 8), rpm range 60 – 200 (opt. 140), salt tolerance up to 4.0 %
AA 33		Small, Round, Biverticillus-spira, Arial mycelium White – Spores grey, pin-pointed, Gram's Positive Temp. range 14 to 37 °C (opt. 28 °C), pH range 5 – 9 (opt. 8), rpm range 60 – 200 (opt. 160), salt tolerance up to 2.0 %
AA 39		Medium, Round, Spira, Arial mycelium White – Spores grey, Dry, Gram's Positive Temp. range 28 to 55 °C (opt. 28 °C), pH range 5 – 11 (opt. 8), rpm range 60 – 200 (opt. 140), salt tolerance up to 6.0 %
AA 48		Small, Uneven, Spira, Arial mycelium White – Spores off-white, Dry, Gram's Positive Temp. range 28 to 55 °C (opt. 28 °C), pH range 3 – 9 (opt. 8), rpm range 60 – 200 (opt. 120), salt tolerance up to 6.0 %
AA 62		Small, Round, Flexibilis, Arial mycelium White – Spores grey, Dry, Gram's Positive Temp. range 4 to 55 °C (opt. 28 °C), pH range 3 – 11 (opt. 8), rpm range 60 – 200 (opt. 140), salt tolerance up to 8.0 %

VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

AA 74		Medium, Round, Flexibilis, Arial mycelium White – Spores grey (color diffused in media – Orange), Dry, Gram's Positive Temp. range 4 to 55 °C (opt. 28 °C), pH range 3 – 11 (opt. 8), rpm range 60 – 200 (opt. 120), salt tolerance up to 6.0 %
AA 83		Small, Round, Spira, Arial mycelium White – Spores White, Flat, Dry, Gram's Positive Temp. range 14 to 37 °C (opt. 28 °C), pH range 5 – 9 (opt. 8), rpm range 60 – 200 (opt. 140), salt tolerance up to 4.0 %
AA 88		Small, Uneven, Flexibilis, Arial mycelium White – Spores White, Dry, Gram's Positive Temp. range 28 to 55 °C (opt. 28 °C), pH range 5 – 11 (opt. 8), rpm range 60 – 200 (opt. 140), salt tolerance up to 4.0 %



	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
AIA Media	6	3	4	0	6	7	2
Soil Extract agar media	3	3	2	2	7	4	3
Starch casein agar media	2	4	2	0	4	4	3
Glucose aspargine agar media	4	2	5	1	5	3	6

Figure 1 Diversity of actinomycetes observed on different media.

3.3 Preliminary screening of actinomycetes isolates for cellulase

The primary screening of actinomycetes isolates for cellulase enzyme was done by spot plate method. After the observation for CI index, among 97 different isolates, isolate AA4, AA19, AA24, AA26, AA33, AA39, AA48, AA62, AA74, AA83 and AA88 showed maximum zone of hydrolysis (CI Index) of 5.00, 6.00, 3.33, 3.00, 3.75, 3.75, 3.45, 3.60, 3.40, 3.02 and 3.54 respectively. Other cellulase positive isolates revealed comparatively lower CI Index. Minimum CI Index 1.00 was estimated from isolate AA50, AA54, AA55, AA56, AA65, AA80, AA95 and AA96 of ninety-seven purified isolates and no zone was observed from isolate AA02, AA03, AA08, AA17, AA20, AA22 and AA36.

3.4 Secondary screening of actinomycetes isolates for cellulase

Among primarily screened out 11 isolates, isolate AA4, AA26, AA33, AA48, AA62 and AA74 showed higher CMCase activities of 3.22 ± 0.03, 2.53 ± 0.11, 2.9 ± 0.08, 2.72 ± 0.02, 3.14 ± 0.02 and 3.06 ± 0.02 U/mL respectively. Isolate AA4, AA19, AA39, AA48, AA62, AA74, AA83, and AA88 showed higher FPase activity of 1.52 ± 0.03, 1.81 ± 0.11, 1.34 ± 0.04, 1.98 ± 0.02, 1.12 ± 0.02, 1.34 ± 0.12 and 1.24 ± 0.02 U/mL respectively. CMCase and FPase activity were much lower in other cellulase-positive isolates. Isolate AA24 yielded a minimum CMCase activity estimate of 1.92 ± 0.02 U/mL, while isolate AA26 yielded an FPase activity estimate of 0.61 ±

VIDYA - A JOURNAL OF GUJARAT UNIVERSITY
Peer-Reviewed, Multidisciplinary & Multilingual Journal
ISSN: 2321-1520 **E-ISSN:** 2583-3537

0.02 U/mL (figure 2). Based on cellulase activity three isolates AA4, AA62 and AA74 were shortlisted for the further work.

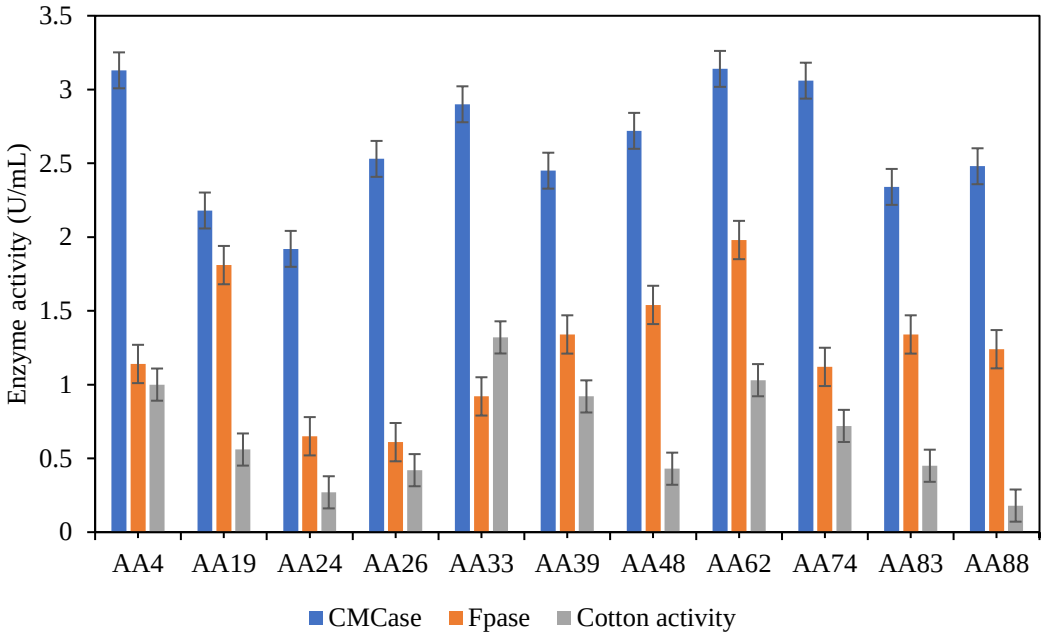


Figure 2 Secondary screening of Actinomycetes.

3.5 Biochemical and Physiochemical characterization of isolates

All three strains are varied with respect to their morphology and arrangements on different ISP medium (Table 3). Tryptone yeast extract, yeast malt agar, Oat-meal medium, Inorganic salt starch, glycerol asparagine, peptone yeast extract iron and tryptone agar media has been used. On all ISP media actinomycetes shows significant growth and biochemical characteristics are listed in table 4. Table 3 Growth characteristics on ISP media

ISP Media		**AA4**		**AA62**		**AA74**	
ISP 1	Tryptone yeast extract medium	G: Good R: Brown	AM: Abundant, White **SP: Yellow brown**	G: Poor R: Grey	AM: Abundant, White SP: None	G: Good R: yellow brown	AM: Abundant SP: None
ISP 2	Yeast malt agar	G: Good R: Brown	AM: Abundant, white **SP: Yellowish**	G: Good R: None	AM: Abundant SP: None	G: Good R: Brown	AM: Abundant **SP: Brown**
ISP 3	Oat-meal medium	G: Good R: None	AM: Moderate, White SP: None	G: Good R: Black	AM: Abundant, White SP: None	G: Good R: none	AM: Moderate SP: None
ISP 4	Inorganic salt starch agar	G: Good R: None	AM: Abundant, white **SP: Yellow brown**	G: Good R: None	AM: Abundant SP: None	G: Good R: None	AM: Abundant, White SP: None
ISP 5	Glycerol asparagine medium	G: Poor R: Yellow	AM: Abundant, grey SP: None	G: Good R: None	AM: Moderate SP: None	G: Good R: Grey	AM: Moderate **SP: Brown**

VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

ISP 6	Peptone yeast extract iron agar	G: Good R: Yellow brown	AM: Abundant, grey SP: None	G: Good R: Grey	AM: Abundant, White SP: None	G: Good R: Black	AM: Abundant, White **SP: black**
ISP 7	Tyrosine agar	G: Good R: Brown	AM: Abundant, grey **SP: Yellow brown**	G: Good R: Grey	AM: Moderate SP: None	G: Good R: Black	AM: Abundant, Grey **SP: Grey**

G: growth of vegetative mycelium; R: reverse side color; AM: aerial mycelium; SP: soluble pigment

Table 4 Biochemical Characteristics of Isolates

Biochemical Characters	AA4	AA62	AA74
Oxidase	–	+	–
lipase	+	+	+
Catalase	+	–	+
Dehydrogenase	–	+	–
Nitrate reduction	+	–	+
Citrate utilization	–	+	+
Voges Proskauer's	–	+	+
Methyl Red	+	+	–
Utilization of carbon source			
Arabinose	–	+	+
Glucose	+	+	+
Lactose	+	+	+
Mannitol	+	+	+
Galactose	–	+	–
Maltose	+	+	+
Fructose	+	+	+
Trehalose	+	+	–
Sucrose	–	+	+
Starch	–	NA	–
Utilization of Nitrogen source			
ONPG	–	+	+
Arginine	+	–	+
Gelatinase	+	–	+
Urease	–	–	+

3.6 Molecular characterization

After being determined to be notable extracellular cellulase producers isolates AA04, AA62, and AA74 were selected for further identification using morphological, biochemical, and molecular characterization techniques. Table 2 and table 3 provides a summary of the morphological features, while Table 4 provides a succinct summary of the biochemical test results. Every isolate had a Gram-positive character. Each isolate had tiny to moderately sized, rounded, rough-textured colonies. All colonies observed as filamentous margin. The colony of isolate AA4 were white in colour and produces white spores, isolate AA62 were off-white in colour and develops grey spores while isolate AA74 were white colony-grey spores and diffuse orange yellow colour in media figure 3. Although isolate AA04 is capable of producing spores, the spore chain disintegrated during the processing of the cultures for SEM (Figure 3 d). AA04 has comparatively straight spore chains rather than coils. One more, isolate AA62 produces spores. The oval spores generate chains, although they don't seem to contain many coils (Figure 3 h). It did not appear that the hyphae and spores had a lot of deposits on them. The hyphae of isolate AA74 are knotted together like ropes, and the spores appear as distinct, spherical particles that are not connected to one another (Figure 3 l).

The isolates were identified using 16S rRNA gene sequencing and BLAST, NCBI similarity search findings as *Pseudonocardia carboxydivorans* AA4 (Accession number – OP764489.1), *Streptomyces griseorubens* AA62 (Accession number – OP679884.1), and *Streptomyces violaceorectus* AA74 (Accession number – OP926029.1). Phylogenetic tree of all isolates was constructed by MEGA version 11.0 [Figure 4 (a), (b), (c)].

VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

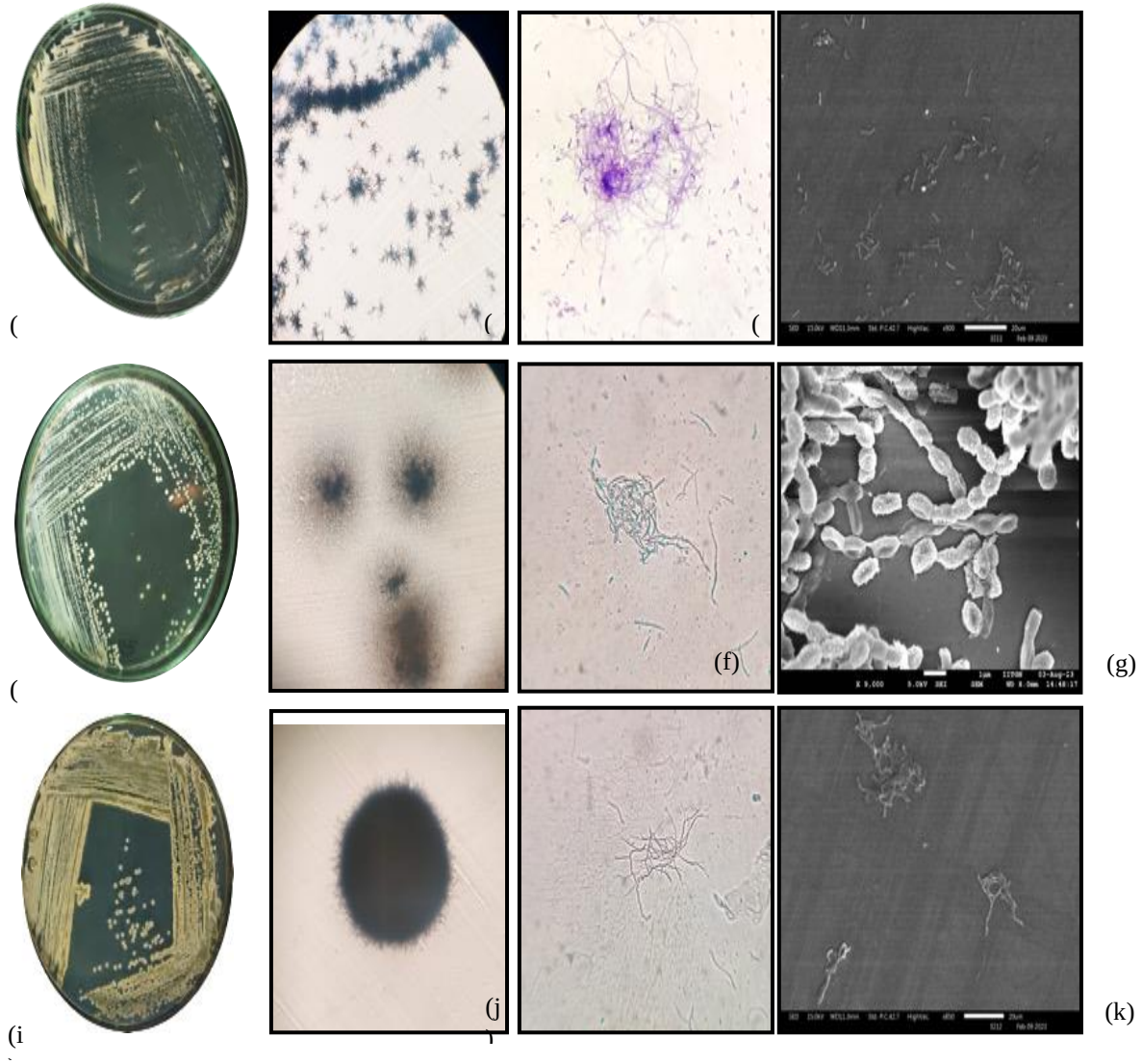
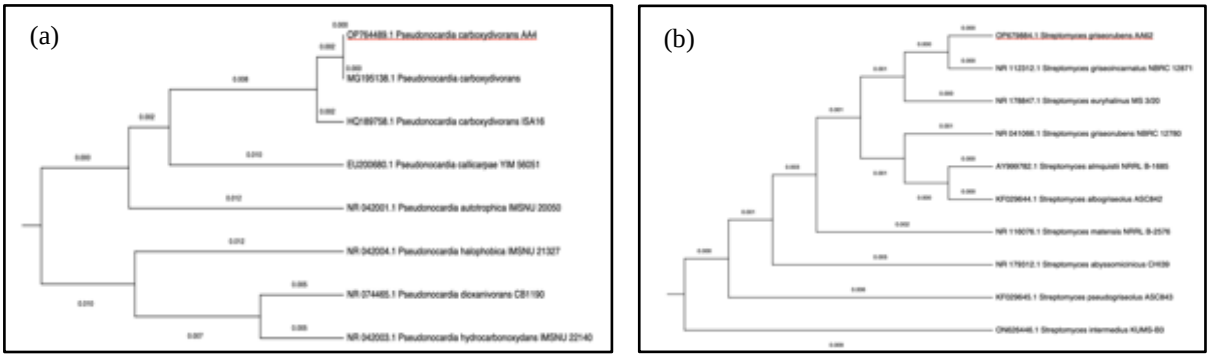


Figure 3 Growth characters, colony morphology, Gram's staining and SEM image of actinomycetes (a) (b) (c) (d) *Pseudonocardia carboxydivorans* AA4 (e) (f) (g) (h) *Streptomyces griseorubens* AA62 (i) (j) (k) (l) *Streptomyces violaceorectus* AA74.

The isolates were identified using 16S rRNA sequencing and BLAST, NCBI similarity search findings as *Pseudonocardia carboxydivorans* AA4 (similarity 100%, Accession number – OP764489.1), *Streptomyces griseorubens* AA62 (similarity 100%, Accession number – OP679884.1), and *Streptomyces violaceorectus* AA74 (similarity 100%, Accession number – OP926029.1). Phylogenetic tree of all isolates is constructed by MEGA version 11.0.



VIDYA - A JOURNAL OF GUJARAT UNIVERSITY

Peer-Reviewed, Multidisciplinary & Multilingual Journal

ISSN: 2321-1520 **E-ISSN:** 2583-3537

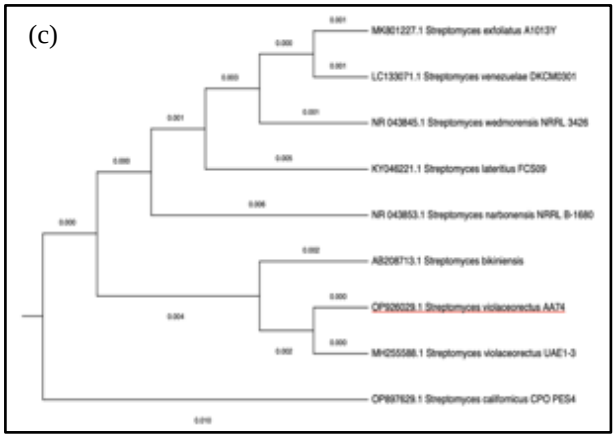


Figure 4 Phylogenetic tree of (a) AA 04 (b) AA 62 (c) AA 74

3.7 Application of *S. griseorubens* AA62 in degradation of liquid cellulosic waste

Among the three cultures tested, *S. griseorubens* AA62 demonstrated the highest CMCase, FPase, and cotton activity. Hence, it was chosen for application purposes. Table 5 illustrates the changes in various physicochemical and biological parameters before and after treatment of a liquid cellulosic waste sample. These changes are significant in evaluating the efficiency of the treatment process.

With the treatment of biomass, the pH decreases from 9.0 to 7.5, indicating degradation of alkaline components during the treatment process which makes effluent safe for environmental release. The transition from an opaque liquid to a transparent one shows the breakdown of suspended particulate matter and removal of turbidity. The blue-colored liquid becomes nearly transparent, indicating the degradation or denaturation of pigments, which is toxic for environmental compliance.

Table 5 Results of treatment of industrial effluent by *S. griseorubens* AA62

	Before treatment	**After treatment**	
		Microbial load	Cell free enzyme
pH	9.0	7.5	8.1
Transparency	Opaque	Transparent	Translucent
Pigment	Blue colored liquid	Nearly transparent	Faint Blue colored
Biological oxygen demand (BOD)	412.73 mg/L	27.12 mg/L	43.21 mg/L
Chemical oxygen demand (COD)	3321.81 mg/L	176.42 mg/L	952.12 mg/L
Cellulose Yield (%)	53.92 %	7.21%	38.02%
Viscosity (cP – centipoise)	1342.61 cP	37.12 cP	153.47 cP

A significant reduction in BOD from 412.73 mg/L to 27.12 mg/L which is in the permissible limit 30.0 – 100.0 mg/L specified by GPCB also observed which demonstrates the breakdown of organic matter that makes the treated water ecofriendly. The COD drops noticeably from 3321.81 mg/L to 176.42 mg/L which is in the permissible limit 250.0 – 1000.0 mg/L specified by GPCB, reflecting the efficient chemical degradation of organic pollutants, including cellulose. The cellulose yield also decreases from 53.92% to 7.21%, indicating effective enzymatic and microbial degradation of cellulose into simpler sugars. The viscosity decreases significantly from 1342.61 cP to 37.12 cP, showing the liquefaction of the effluent and breakdown of cellulose.

The effluent was treated using a cell-free enzyme, resulting in a pH of 8.1 after treatment. The effluent remained colored but became translucent. Additionally, the biological oxygen demand (BOD) and chemical oxygen demand (COD) were reduced to 43.21 mg/L and 952.12 mg/L, respectively which in the range of permissible limit specified by GPCB. The cellulose yield decreased to 38.02%, and the viscosity was recorded to 153.47 cP. Which shows Biomass was more effective with compared to cell free enzyme.

CONCLUSION

This study revealed the significance of culture dependent diversity of the soil actinomycetes of the Gujarat Region. Various physicochemical factors of soil influencing the actinomycetes diversity were analyzed, reflecting the species richness and evenness. Besides the systematic study of actinomycetes, the cellulolytic and antimicrobial potentials of identified actinomycetes suggest significant waste bioremediation avenues.

ACKNOWLEDGEMENTS

All authors acknowledge the support provided by Department of Microbiology and Biotechnology. Aanal Patani is thankful to ScHeme of Developing High quality research (SHODH), Education department, Government of Gujarat, India for providing student support fellowship.

ISSN: 2321-1520 **E-ISSN:** 2583-3537

We are not receiving any fund for the research from any funding agency.

REFERENCE

1. Behera BC, Sethi BK, Mishra RR, Dutta SK, Thatoi HN. Microbial cellulases – Diversity & biotechnology with reference to mangrove environment: A review. J Genet Eng Biotechnol. 2017;15(1):197-210. doi:10.1016/J.JGEB.2016.12.001
2. Chaudhari, P. R., Desai, N. H., Chaudhari, P. P., & Rabari, K. V. (2018). Status of chemical properties and available major nutrients in soils of Patan district of Gujarat, India. *Crop Research*, *53*(3and4), 147-153. http://doi.org/10.31830/2454-1761.2018.0001.9
3. Castillo, U., Myers, S., Browne, L., Strobel, G., Hess, W. M., Hanks, J., & Reay, D. (2005). Scanning electron microscopy of some endophytic streptomycetes in snakevine-Kennedia nigricans. *Scanning*, *27*(6), 305-311. https://doi.org/10.1002/sca.4950270606
4. Al_husnan, L. A., & Alkahtani, M. D. (2016). Molecular Identification of Streptomyces producing antibiotics and their antimicrobial activities. *Ann. Agric. Sci*, *61*(2), 251-255. https://doi.org/10.1016/j.aoas.2016.06.002
5. Wei, J., He, L., & Niu, G. (2018). Regulation of antibiotic biosynthesis in actinomycetes: perspectives and challenges. *Synth. Syst. Biotechnol.*, *3*(4), 229-235. https://doi.org/10.1016/j.synbio.2018.10.005
6. Biswal, T., BadJena, S. K., & Pradhan, D. (2020). Sustainable biomaterials and their applications: A short review. *Mat. Today: Proceedings*, *30*, 274-282. https://doi.org/10.1016/j.matpr.2020.01.437
7. Hammes, F., & Verstraete*, W. (2002). Key roles of pH and calcium metabolism in microbial carbonate precipitation. *Rev. Environ. Sci. Biotechnol.*, *1*, 3-7.
8. Crowther, T. W., Van den Hoogen, J., Wan, J., Mayes, M. A., Keiser, A. D., Mo, L., ... & Maynard, D. S. (2019). The global soil community and its influence on biogeochemistry. *Science*, *365*(6455), eaav0550. DOI: 10.1126/science.aav0550
9. Bhatla, S. C., A. Lal, M., Kathpalia, R., & Bhatla, S. C. (2018). Plant mineral nutrition. *Plant Physiol. Dev. Metab.*, 37-81.
10. Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. J. *Trop. Med.*, *2019*. https://doi.org/10.1155/2019/1895340
11. Malviya, M. K., Pandey, A., Sharma, A., & Tiwari, S. C. (2013). Characterization and identification of actinomycetes isolated from 'fired plots' under shifting cultivation in northeast Himalaya, India. *Ann. Microbiol.*, *63*, 561-569. DOI 10.1007/s13213-012-0504-x
12. Shivlata, L., & Satyanarayana, T. (2015). Thermophilic and alkaliphilic Actinobacteria: biology and potential applications. *Front. Microbiol.*, *6*, 1014. https://doi.org/10.3389/fmicb.2015.01014
13. Pradeep, N. S., & Edison, L. K. (Eds.). (2022). *Microbial Beta Glucanases: Molecular Structure, Functions and Applications*. Springer Nature.
14. Sapkota, A., Thapa, A., Budhathoki, A., Sainju, M., Shrestha, P., & Aryal, S. (2020). Isolation, characterization, and screening of antimicrobial-producing actinomycetes from soil samplesInt. J. Microbiol., *2020*. https://doi.org/10.1155/2020/2716584
15. Aanal, P., Apoorva, V., Jahanvi, T., Jaimin, P., Pooja, S., Devanshi, V., Rashmilata, C., Vikram, R., Kiransinh, R., Rakesh, P. (2023). Exploration of soil microflora for biosynthesis of cellulases. *Advances in Biotechnology, 2023*. DOI: 10.15515/abr.0976-4585.S1.19
16. Siva, D., Srivethi, G., Vasan, P. T., Rajesh, D., Alfarhan, A., & Rajagopal, R. (2022). Enhanced cellulase enzyme production by Aspergillus niger using cellulase/iron oxide magnetic nano – composites. *J. King Saud Univ. - Sci.*, *34*(1), 101695. https://doi.org/10.1016/j.jksus.2021.101695
17. Saini, A., Aggarwal, N. K., & Yadav, A. (2017). Isolation and screening of cellulose hydrolyzing bacteria from different ecological niches. Bioeng. Biosci., *5*(1), 7-13. DOI: 10.13189/bb.2017.050102
18. Zhu, H., Sandiford, S. K., & van Wezel, G. P. (2014). Triggers and cues that activate antibiotic production by actinomycetes. *J. Ind. Microbiol. Biotechnol.*, *41*(2), 371-386. https://doi.org/10.1007/s10295-013-1309-z
19. Busti, E., Monciardini, P., Cavaletti, L., Bamonte, R., Lazzarini, A., Sosio, M., & Donadio, S. (2006). Antibiotic-producing ability by representatives of a newly discovered lineage of actinomycetes. Microbiology, 152(3), 675-683. http://doi.org/ 10.1099/mic.0.28335-0
20. Kumar, S., Solanki, D. S., Parihar, K., Tak, A., Gehlot, P., Pathak, R., & Singh, S. K. (2021). Actinomycetes isolates of arid zone of Indian Thar Desert and efficacy of their bioactive compounds against human pathogenic bacteria. *Biol. Futura.*, *72*(4), 431-440. https://doi.org/10.1007/s42977-021-00073-5
21. Saravanakumar, A., Rajkumar, M., Serebiah, J. S., & Thivakaran, G. A. (2008). Seasonal variations in physico-chemical characteristics of water, sediment and soil texture in arid zone mangroves of Kachchh-Gujarat. *J. Environ. Biol*, *29*(5), 725-732.s

ISSN: 2321-1520 **E-ISSN:** 2583-3537

22. THAKER, P. N., BRAHMBHATT, N., & SHAH, K. D. (2023). Assessment of physico-chemical properties of soil in selected areas of Khambhat taluka, Gujarat. *Ann. Plant Soil Res.*, *25* (1), 187-192. https://doi.org/10.47815/apsr.2023.10255
23. Kumar, N., Singh, R. K., Mishra, S. K., Singh, A. K., & Pachouri, U. C. (2010). Isolation and screening of soil Actinomycetes as source of antibiotics active against bacteria. *Int. J. Microbiol. Res.*, *2*(2), 12.
24. Clarridge III, J. E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical microbiology reviews*, *17*(4), 840-862. https://doi.org/10.1128/cmr.17.4.840-862.2004
25. Darby, A. C., Chandler, S. M., Welburn, S. C., & Douglas, A. E. (2005). Aphid-symbiotic bacteria cultured in insect cell lines. *Applied and environmental microbiology*, *71*(8), 4833-4839**.** https://doi.org/10.1128/AEM.71.8.4833-4839.2005
26. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of molecular biology*, *215*(3), 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2
27. Shirling, E. T., & Gottlieb, D. (1966). Methods for characterization of Streptomyces species. *International journal of systematic bacteriology*, *16*(3), 313-340.
28. Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120