# Isolation and Characterization of Cellulase Producing *Streptomyces albaduncus* from Gut of *Eisenia foetida*

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**Abstract**— Cellulose is the most abundant biopolymer on earth, recognized as the major component of plant biomass. The cellulolytic microorganisms were isolated from gut of earthworm (Eisenia foetida). Using Glycerol asparagine agar media, the organisms were identified as Actinomycetes sps.. The endoglucanase activity of the culture broth was determined during actinomycetes growth by measuring the release of reducing sugar from carboxyl methyl cellulose, mineral salts medium. The reducing sugar was assayed by the dinitro salicylic acid method, using glucose ass the sugar standard. One unit of cellulose was defined as the amount of enzyme which produced one micro mole glucose equivalent per minute under the assay condition. The production of enzyme in Mineral salts medium was studied for the effect of temperature and pH. The production of cellulose enzyme was low and at $37^0$C to $50^0$C optimum was observed. The optimum production of cellulose was observed at pH of 6.5-7.0.

**Index Terms**— *Streptomyces albaduncus*, *Eisenia foetida, cellulolytic enzyme, carboxy methyl cellulose, EGAS 1(Earthworm gut actinomycetes), ISP (International strepotomyces project)*

## 1 INTRODUCTION

Green revolution had played a significant role in Indian agriculture with the introduction of chemicals, facilitating the country to be self reliant in crop production. Soon, several reviews had revealed that the continuous use of chemical fertilizers in high doses that are easily soluble had contributed to soil degradation, through a combined effect of acidification, micro nutrient depletion, ground and surface water pollution and reduced soil microbial activity, etc. Now there is a growing realization that the adoption of ecological and sustainable farming practices can reverse the declining trend in the global productivity and environmental protection. The growing concern about the adverse affects of chemicals in agriculture has led to the development of ecoagriculture that encourages the use of organic inputs like biofertilizers and biopesticides etc., to produce better quality organic products while protecting the soil health. The usual practice of disposing the agrowastes by the farmer is burning or dumping as farmyard manure resulting in the loss of nutrients and causing environmental pollution. Use of the microbial populations in consortia will help to recover the nutrients by their enzymatic processes. Cellulose is considered as one of the most important sources of carbon on this planet. Its annual biosynthesis by both land plants and marine algae occurs at a rate of $0.85x\ 10^{11}$ tons per annum. Cellulose degradation and its subsequent utilization is important for global carbon sources. The value of cellulose as a renewable source of energy has made cellulose hydrolysis as the subject of intense research and industrial interest.

The role of microbial activity in the earthworm gut, cast and soil is very essential for the degradation of organic wastes for the release of nutrients to plants. During vermicomposting organic matter undergoes, physico-chemical and bio-chemical changes by the combined effect of earthworm gut flora and also other microbial activities. Earthworm transforms the constituents of organic waste into a more useful vermicompost initially by grinding and digestion by aerobic and anaerobic microflora. Much of the research on vermicomposting had been focused on the changes in the chemical parameters.

*Eisenia foetida* is the universally accepted species for vermicomposting; hence it is the chosen invertebrate for this study. Earthworms are eco-friendly and play a variety of roles in agro ecosystem. The gut of earthworm is the factory to manufacture the beneficial microbial densities and their products. The excreted nutrients enrich thousand times more than the surrounding soil. Experiments have proven that crops grown in earthworm inhabitant soils had increased the yields from 25% to over 300% than in earthworm free soils (Barley, 1961). Microbial community indigenous to the earthworm *Eisenia foetida* was studied. The earthworm gut is favorable for the development of actinomycetes due to neutral pH, optimal humidity and temperature. In addition, the increased organic carbon and nitrogen content in earthworm gut may also stimulate microbial activity.

In previous studies, some enzymes (e.g. amylase, protease, phosphatase nitrogenase and cellulase) which decompose soil organic materials were detected in the gut contents of earthworm converting the unavailable forms to available for promotion of plant growth. However, their interactions with soil microorganisms are still poorly understood, in particular the effect of gut passage on the community structure of ingested microorganisms. Moreover, it is still unsolved, if earthworms, like many other soil-feeding invertebrates, possess an indigenous gut microbial community. A large number of microorganisms are capable of degrading cellulose. Cellulases for native cellulose are commercially produced only with strains of the fungus *Trichoder-*

*ma* and few bacteria like *Clostredium thermocellum* etc,. Actinomycetes like *Streptomyces sp*. and *Thermoactinomyces* have also been reported to produce cellulase. Actinomycetes have characteristics, which are transitional between bacteria and fungi and are sometimes called fungi like bacteria. Growth of actinomycetes is hyphal and they may form a mycelia characteristic of the fungi. Actinomycetes are identified by their aerial mycelia and characteristic conidia that differentiate from fungal species. Actinomycetes are more abundant in dry soils than in wet soils and more in cultivated soils and compost. They are responsible for the decomposition of the more resistant organic matter of soil and produce a number of dark black to brown pigments, probably contributing to the dark colour of soil humus.

## 2 MATERIALS AND METHODS

### 2.1 Collection of Earthworm Gut homogenate

*Eisenia foetida*, the earthworm is collected from vermicompost pits from S.V.Agricultural University, Tirupati. Healthy adult earthworms were collected and allowed to starve for 24 hours. The earthworms were then disinfected with 70% ethanol and gut was dissected out. The content was weighed and homogenised (for 5 minutes in a vortex mixer) in 0.85% NaCl solution (Toyoto and Kimura, 2004). Further, the isolation was by dilution plate method (Cuppucino and Sharmen, 2008).

### Isolation of cellulose degrading actinomycetes from the gut of earthworm

The gut homogenate was serially diluted ($10^{-1}$-$10^{-8}$) and centrifuged at 500 rpm for 20 minutes to disperse the spore chains. The suspension was allowed to settle for 1hr and plated on to Glycerol Asparagine Agar (GAA) ISP 5 [*International Streptomyces Project* (ISP; Shirling and Gottlieb, 1966)]. Three replicates were considered for each dilution. The same suspension was plated on to Starch Casein Agar (SCA) (Okazami and Okami, 1972). The SCA was supplemented with cyclohexamide, streptomycin and nystatin of each 50 µg/ml to inhibit the normal bacterial and fungal flora. The plates were incubated at $30^0$C and observed for 7 days for the growth and sporulation of actinomycetes. The pure cultures were maintained on GAA (ISP5) at $4^0$C.

### SCREENING OF THE ISOLATES FOR CELLULOLYTIC PROPERTY

The five actinomycetes isolates obtained were screened for their cellulolytic activity on Cellulose agar medium using Carboxy Methyl Cellulose (CMC), a chromogenic substrate source. Colonies were selected for further studies based on the formation of larger clearing zones on the plates with Carboxy Methyl Cellulose and were designated as Earthworm Gut Actinomycetes (EGAS) and assigned numbers from 1-5.

## CHARACTERIZATION FOR THE TAXONOMIC POSITION OF THE SELECTED CELLULOLYTIC ACTINOMYCETES ISOLATE (EGAS1)

To characterize the taxonomic position of the selected EGAS1 isolate, a range of tests were carried for morphological, physiological and biochemical out according to the guidelines of Bergey's Manual of Systemic Bacteriology (Volume IV, Locci, 1989), *International Streptomyces Project* (Shirling and Gottlieb, 1966) and Williams *et al.*, (1983).

**Morphological Characteristics**

***Gram's staining***

A loopful of EGAS1 culture grown in GAA was smeared and heat fixed. The smear was covered with crystal violet for 30 seconds, then washed with distilled water for a few seconds and flooded with Gram's iodine solution. After 30 seconds, the slide was washed and decolorized with 95% (v/v) ethyl alcohol. The slide was slightly washed with distilled water and counter stained with saffranine solution for 30 seconds and observed under microscope.

***Spore chain morphology***

Spore mass color, elevation, spore shape, spore surface and its position were recorded. The species belonging to the actinomycetes genera are grouped into three sections with regard to the spore chains (Shriling and Gottlieb, 1996), namely rectiflexibiles (RF), retinaculiaperti (RA) and spirales (S). When strain forms two types of spore chains, both were noted as RAS. This was determined by performing Phase contrast microscopy for 14 day old culture grown on Glucose Yeast extract - Malt extract agar (GYMA) ISP2 (Shriling and Gottlieb, 1996).

***Ariel mass colour:***

The mature aerial mycelium of EGAS1 was observed for White, Yellow, Gray, Red, Green, Blue and Violet coloration.

***Reverse side pigments***

EGAS1 was studied for its ability to produce characteristic pigments on the reverse side of

the colony and described as distinct (+ve), (Red, Orange, Green, Blue or Violet) and not distinctive (-ve) or none (Yellow, Brown, Olive or Yellowish brown).

***Diffusible pigments:***

EGAS1 was studied for its ability to produce soluble pigments into the medium after incubation for 14 days. The pigmentation was described as producer (+) or not producer (-). The color was recorded as red, orange, green, yellow, blue and violet. pH sensitivity of the diffusible pigments was assessed by noting any colour change induced in the medium by the addition of acid or alkali (Shriling and Gottlieb, 1996).

***Melanin pigment production***

Based on the production of melanoid pigments i.e., Greenish brown, Brownish black or Distinct brown colours in the medium for modified pigment and the strains were grouped as melanoid pigment producer (+ve) or not producer (-ve). This was determined after 5 days of incubation of the EGAS1 culture on Peptone yeast extract iron agar and Tyrosine agar (Shriling and Gottlieb, 1996).

**Physiological tests**

## *Growth characteristics*

Growth characteristics of the culture were observed using different types of media specified by *International Streptomyces Project* (ISP) (Shirling and Gottlieb, 1966) such as ISP2 (Glucose Yeast Extract - Malt Extract Agar), ISP3 (Oatmeal Agar) ISP5 (Glycerol Aspargine Agar), ISP6 (Peptone Yeast Extract Iron agar), ISP7 (Tyrosine agar) Starch Casein Agar (SCA) and Nutrient agar.

***Utilization of Carbon source***

Different carbon sources with 1% (w/v) of D- Mannitol, L- Rhamnose, Maltose, Glucose, Lactose, Raffinose and Sucrose were incorporated into Basal medium containing nitrogen source and pH was adjusted to 7.0 (Williams, 1983).

EGAS1 was inoculated on to this medium and incubated at $30^0$C for 5 days. The ability of the strain to use a carbon source was determined by comparing the growth of the strain with that of the unsupplimented basal medium set up as negative and a positive control containing D-glucose (1% w/v).

***Utilization of Nitrogen source***

The ability of the strain to utilize nitrogen sources several aminoacids like L-Aspargine,L-Alanine, L-Arginine and L-Histidine was tested by incorporating each source of nitrogen at 1% (w/v) level, using the basal medium containing glucose at pH 7.0. The plates were inoculated with the organism and incubated for 5 days at $30^0$C. Utilization of the nitrogen source was determined by comparison of the growth with that in the positive control using L-Asparagine (1% w/v) and the negative control with no nitrogen source.

## Resistance to antibiotics

EGAS1 was tested for its ability to grow in the presence of some antibiotics. Filter paper discs with known concentration of different antibiotics (Himedia) like Penicillin-G, Amphicillin, Streptomycin, Erythromycin, Ciprofloxacin and Chloramphenicol of each 100 µg/ml were placed on the surface of the medium inoculated with 0.1 ml of EGAS1 spore suspension. Definite inhibition zone was noted after 72 hrs of incubation and recorded as susceptible i.e., positive, resistant or negative and intermediate.

***Growth at different temperatures***

To detect the influence of temperature on the growth of the EGAS1 at different temperatures, the freshly inoculated Glycerol Asparagine Broth (GAB) was incubated at $4^0$C, $10^0$C, $15^0$C, $25^0$C, $30^0$C, $35^0$C, $40^0$C, $45^0$C, $50^0$C and $55^0$C for 5 days. To determine the optimum temperature for the growth of the organism was recorded in terms of absorbance at 540 nm.

***Growth at different concentrations of NaCl (%)***

The growth of the EGAS1 isolate was detected by inoculating fresh cultures on GAB containing 1%, 2%, 4%, 6%, 8% and 10% of NaCl and incubated at $30^0$C for 5 days. The broth was read at the absorbance of 540 nm to determine optimum NaCl level for the growth

***Growth at different pH***

The growth of the isolate was detected at pH 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 by inoculating fresh cultures on GAB and incubated at $30^0$C for 5 days for EGAS1 and the growth was recorded by reading the absorbance at 540 nm.

**Biochemical tests**

For the isolate EGAS1, different biochemical tests were conducted including utilization of sugars and amino acids according to the guidelines of Bergey's Manual of Systemic Bacteriology Vol. IV. (Locci, 1989, Cappuccino and Sherman, 2008, Williams *et. al*., 1983, Mackie and McCartney, 1989).

**Enzyme Activities**

***Oxidase test***

Single colony of EGAS1, grown on Glycerol asparagine agar was spread with a glass rod on oxidase strip (Whatman No.1 filter paper strips and soaked in a freshly prepared 1% solution of tetramethyl-p-phenylene-diamine dihydrochloride) to detect the presence of oxidase by the change in colour of the strip to violet colour within 5-10 seconds to indicate positive test or no colour change to indicate negative.

***Catalase test***

To observe for catalase production, 5 days culture of EGAS1 was added with 1ml of hydrogen peroxide (37%) on a glass slide and observed for the production of effervescence.

***Urease test***

The production of urease by the EGAS1 isolate was tested by growing the organism on Christensen's urea agar slants. The tubes were incubated at $30^0$C for 5 days. Urease activity was indicated by the production of ammonia with a changes in the medium from yellow to pink colour which was indicated as positive reaction.

**IMViC Tests**

***Indole test***

A loopful of EGAS1 isolate was inoculated in to peptone broth and incubated for 5 days at $30^0$C. The production of indole was tested by adding 10 drops of Kovac's reagent (dimethyl aminobenzaldehyde) and formation of cherry red colour in the alcohol layer to demonstrate the formation of Indole from tryptophan.

***Methyl red test***

Ability of the EGAS1 isolate to produce acid by fermenting glucose present in Glucose Phosphate Peptone water (GPP) medium was tested by adding five drops of 0.04% methyl red indicator to 5 day old culture tube and results were recorded immediately. Appearance of bright red color in the medium indicates a positive reaction.

***Voges-Proskauer test***

A loopful of EGAS1 isolate was inoculated in to GPP water and incubated for 5 days at $30^0$C. Development of a deep rose colour within 15 min following the addition of Barritt's reagent (10 drops) was indicative of the presence of acetyl methyl carbinol. The absence of rose colouration was indicated as negative result.

***Citrate utilization***

The EGAS1 isolate was streaked on to Simmon's Citrate medium (pH 6.8) and incubated at $30^0$C for 5 days for detecting the ability to utilize citrate as sole source of carbon and ammonium salt as the source of nitrogen. Cultures were observed for the presence of growth accompanied by change in colour to blue. The media that remain green without any growth will be citrate negative.

***$H_2S$ production***

It was determined by inserting saturated lead acetate strips in to the neck of the peptone water culture tubes. Any blackening after incubation at $30^0$C for 5 days was recorded as positive result and no colour change as negative.

***Nitrate reduction test***

The production of nitrate reductase enzyme was tested by inoculating the culture into nitrate broth and incubating for 5 days at $30^0$C. The freshly mixed 0.1 ml test reagent containing sulfanilic acid and alpha naphthylamine in acetic acid was added. Development of red colour within a few minutes indicates the presence of nitrite resulting from reduction of nitrate.

**Utilization of sugars**

The ability of the EGAS1 isolate to ferment different carbohydrates was tested. The different carbohydrates used were D-mannitol, rhamnose, maltose, glucose, fructose and raffinose. Peptone water was supplemented with 0.5% of each sugar, Andrade's indicator (0.005%) was added to the medium with Durham tubes. The tubes were inoculated with the EGAS1 isolate and incubated at $30^0$C for 5 days and acid/gas production was recorded.

**Utilization of nitrogen sources**

***Arginine dihydrolase test***

The production of ammonia from Arginine catalyzed by Arginine dihydrolase was tested by growing the isolate for 5 days at $30^0$C, in liquid broth supplemented with 1% L-arginine hydrochloride and 0.002% phenol red.

***Gelatin hydrolysis***

The EGAS1 isolate was inoculated onto the gelatin agar plates and incubated at $30^0$C for 5 days. Mercuric chloride solution (1.0% w/v) was flooded over the culture and allowed to stand for 5 to 10 min and observed for liquefaction and formation of clear halo around the growth of the organism indicates positive result.

***Starch hydrolysis***

Amylase production by EGAS1 isolate was detected by spot inoculation onto the starch agar plates with a single colony. The plates were incubated at $30^0$C for 5 days. Gram's iodine solution was flooded on to the surface of the plates and allowed to stand for 30 seconds and poured of excess, a clear zone of hydrolysis around the growth of the organism was indicative of positive result.

***Casein hydrolysis***

The EGAS1 isolate was spot inoculated onto the Skimmed milk agar and incubated at $30^0$C for 5 days. After incubation the plates were observed for the clear zone of hydrolysis around the colonies.

***Tween 20 hydrolysis***

EGAS1 isolate was spot inoculated on to Tween 20 agar medium (1% w/v) and incubated for 5 days at $30^0$C. Clear zone of lysis around the organism is indicative of positive reaction.

***Esculin hydrolysis***

Esculin hydrolysis was determined by spot inoculation of the EGAS1 isolate on to Esculin agar medium (0.1% w/v) and its incubation for 5 days at $30^0$C. Clear zone of lysis around the growth of the organism indicates its hydrolysis.

***Tyrosine hydrolysis***

Tyrosine hydrolysis was determined by spot inoculation of the EGAS1 isolate on to Tyrosine Agar medium and incubated for 5 days at $30^0$C. Clear zone of lysis around the organism indicates its ability to hydrolyze Tyrosine.

**PRODUCTION OF CELLULASE BY EGAS1 ISOLATE**

Mineral salts medium (100 ml in 500 ml Erlenmeyer flask) was inoculated with 1ml of 5 day old EGAS1 culture and incubated at $30^0$C with vigorous aeration in a shaker at 150 rpm for 7 days. Cells were separated by centrifugation at 8,000 x g for 20 minutes at $4^0$C. The cell free culture filtrate was used as crude enzyme source. Filter paper assay method was employed to determine cellulase activity of the filtrate (Mandels and Weber, 1976). Filter paper activity was a measure of total cellulolytic activity resulting from combined action of different enzyme components present in the culture filtrate. In this method, the Whatmann No. 1 filter paper as a cellulosic substrate was incubated with EGAS1 isolate as a source of enzyme. The liberation of reducing sugar was measured by DNS method. One unit of cellulase was defined as the amount of enzyme releasing 1 Micromole of reducing sugar per ml per minute. Protein concentration was measured by Lowry method (1951).

## RESULTS AND DISCUSSION

### Isolation and Screening for cellulose degrading actinomycetes from the gut of earthworm

A total of 5 Actinomycetes isolates designed as EGAS 1-5 were obtained from earthworm (*Eisenia foetida*) gut homogenate on incubation for 5 days at $30^0$C on Glycerol Asparagine Agar medium. In the present study all the 5 isolates were subjected for their cellulolytic property on CMC agar. Among the 5 isolates (EGAS1-5), efficient actinomycetes isolate was selected according to its highest cellulolytic activity and on the basis of its growth. Good cellulolytic activity was exhibited by an isolate and was designated as EGAS1 (Fig 2). The cellulolytic activity was measured by diameter of zone of clearance in mm and it was noted as 23 mm for EGAS1.

## CHARACTERIZATION FOR THE TAXONOMIC POSITION OF THE SELECTED ACTINOMYCETES (EGAS1) ISOLATE

**Morphological, physiological and biochemical characteristics of *EGAS1***

Taxonomic study of EGAS1 isolate had shown the morphological, physiological and biochemical characteristics typical of *Streptomyces* (Table 1). EGAS1 was characterized according to the guidelines given by Shirling and Gotllieb (1966) (*International Streptomyces project*), Williams *et al.*, (1983) and Bergey's Manual of Systemic Bacteriology, Vol. IV (1989). Growth characteristics of EGAS1 was observed using different types of media such as ISP2 (Malt Yeast Extract Agar), ISP3 (Oatmeal Agar), ISP5 (GYMA), ISP6 (Peptone Yeast Extract Iron Agar), ISP7 (Tyrosine Agar), SCA, GAA and Nutrient Agar (NA). EGAS1 had exhibited good growth on GAA, SCA and NA (Fig. 3) moderate growth on MYEA , OA and

GYMA and no growth was recorded on ISP6 and ISP7.

**Physiological characteristics**

The effect of temperature from $4^0$C – $55^0$C was studied on the growth of EGAS1 with intervals of $5^0$C for 5 days. It was found to be optimum at $30^0$C and showed temperature tolerance up to $45^0$C. The strain was grown on GAA at pH value 5 – 11 for 5 days and found that good growth was observed at pH 5.5. Growth was observed in the range of 1-10% NaCl concentration and 4% NaCl concentration was found optimum for growth. Diffusible pigments were not produced. On the reverse side of the culture, the pigment of the colony was yellow (Fig. 3). The spore mass of EGAS1 was found to be white to gray in color and it is central. All spores showed smooth to warty surface and shape varied from oval to rod shape with bulging sporangia.

At all stages of growth the cells were found as Gram positive long rods. Carbon, Nitrogen utilization pattern and other biochemical tests were performed according to standard methods described for actinomycetes. Strain EGAS1 had used D-mannitol, Rhamnose, Maltose and Glucose for growth. However Raffinose, Lactose and Sucrose were not utilized Acid was not formed from these carbon sources. L-Aspargine, L-Alanine, L-Histidine and L-Arginine were utilized as nitrogen source for growth. Tests for Catalase, Oxidase and Urease were positive. For IMVIC tests EGAS1 was detected to be negative for Indole, positive for Methyl red (Fig 4) and negative for Citrate utilization and Voges-prausker test (Table 1). Nitrate was not reduced to Nitrite. Gelatin was liquefied, $H_2S$ production was negative. It was resistant to Ampicillin, Penicillin-G and Streptomycin but sensitive to Erythromycin, Ciprofloxacin and Chloramphenicol. An array of enzymes were produced by the organism and Casein, Starch, Gelatin, Tween 20 and Urea were hydrolysed.

**Table 1: Morphological, physiological and biochemical characteristics of EGAS1.**

| Characteristics | |
|---|---|
| **MORPHOLOGICAL CHARACTERISTICS** | |
| **Cultural characteristics** | |
| • Margin | Regular |
| • Elevation | Convex |
| • Surface | Dull |
| • Pigment | -- |
| • Opacity | Opaque |
| • Motility | - |
| • Fluorescence | - |
| • Reverse side pigments | Yellow to brown |
| • Diffusible pigments | - |
| • Melanin pigments production | + |
| • Ariel mycelium | - |
| **Spore morphology** | |
| • Gram's reaction | +ve |
| • Cell shape | long rods |
| • Spore mass | Grey |
| • Endospore | + |
| • Position | Central |
| • Spore Shape | rod shape |
| • Sporangia bulging | + |
| • Spore surface | Smooth to warty |
| • Spore chain morphology | Retinaculiaperti |
| **PHYSIOLOGICAL CHARACTERISTICS** | |
| ***Temperature range*** | |
| • $4^0$C | - |
| • $10^0$C | - |
| • $15^0$ C | - |
| • $25^0$ C | - |
| • $30^0$ C | + |
| • $35^0$C | + |
| • $40^0$C | + |
| • $45^0$C | + |
| • $50^0$ C | - |
| • $55^0$ C | - |
| • Optimum temperature | $30^0$C |
| **pH range** | |
| • pH 5.0 | + |
| • pH 6.0 | + |
| • pH 7.0 | + |
| • pH 8.0 | + |
| • pH 9.0 | + |
| • pH 10.0 | + |
| • pH 11.0 | + |
| • Optimum pH | 5.5 |
| **NaCl range (%)** | 1-10% |
| • 1.0 | + |
| • 2.0 | + |
| • 4.0 | + |
| • 6.0 | + |
| • 8.0 | - |
| • 10.0 | - |
| • Optimum NaCl conc. | 4% |
| **BIOCHEMICAL TESTS** | |
| ***Degradation/Hydrolysis of*** | |
| • Casein | + |
| • Cellulose | + |
| • Starch | + |

- Gelatin +
- Tween 20 +
- Urea +

Enzymatic activity

- Catalase activity +
- Oxidase activity +
- Urease activity +

IMVIC Tests

- Indole test -
- Methyl Red test +
- Voges-Proskauer test -
- Citrate Utilization -
- Hydrogen sulphide Test -
- Nitrate reduction -

## Utilization of sugars:

- D- Mannitol +
- L-Rhamnose +
- Maltose +
- Glucose +
- Lactose -
- Raffinose -
- Sucrose -

**Utilization of Nitrogen sources:**

- L- Aspargine +
- L- Alanine +
- L- Histidine +
- L-Arginine +

**Resistance to Antibiotics**

- Pencillin-G +
- Amphicillin +
- Streptomycin +
- Erythromycin -
- Ciprofloxacin -
- Chloramphenicol -

+ Positive, - Negative

**Table 2: Culture characteristics of EGAS1 on different media**

| Medium | Growth |
|---|---|
| **Glycerol Aspargine agar (GAA)** | Good |
| **Starch Casein Agar (SCA)** | Good |
| **Nutrient Agar (NA)** | Good |
| **Malt – Yeast Extract Agar (MYEA)** | Moderate |
| **Oatmeal Agar (OA)** | Moderate |
| **Glucose-Yeast-Malt extract Agar (GYMA)** | Moderate |
| **Tyrosine Agar** | No growth |
| **Peptone Yeast Extract Iron Agar** | No growth |

Based on the morphological, cultural and biochemical characteristics the organism EGAS1 was identified and characterized as *Streptomyces albaduncus* with the assistance of IMTECH, Chandigarh, India.

**CELLULASE PRODUCTION BY *Streptomyces albaduncus* (EGAS1)**

After identification of actinomycete culture, the efficiency of the organism for cellulase production was determined using the Mineral Salts Medium. Cellulolytic activity of EGAS1 isolate was determined according to filter paper assay method (Mandels & Weber, 1969). The amount of soluble reducing sugar that was (glucose) released into the production medium was determined. The cellulase activity was expressed in terms of Filter Paper Units (FPU). The volume of EGAS1 isolate filtrate responsible for the release of 1µ mole of glucose per min was considered to be one filter paper unit. Since *Streptomyces albaduncus* had been detected to exhibit highest cellulolytic activity in terms of filter paper units of 1.92 FPU/ml, this organism was further exploited to assess the cellulolytic potential in subsequent experiments.

## Conclusion:

Bio-inoculants of the earthworm gut flora have to be further eplored to replace chemical fertilizers, in view of earthworm becoming a rarity.The technology should be tesed further and simplified, so that farmer can adopt this knowledge for the benefit of the crop economics.

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## REFERENCES

[1] K.P. Barley, Advances in Agronomy. 13: 262-264. 1961.
[2] J.G. Cappucino and N.Sherman, Denitrification. In Microbiology a laboratory manual. International student edition. 4th edition. Addition Wesely Publication. P. 313.1999.
[3] R. Locci, Streptomyces and related genera. In : Bergey's Mannual of systemic bacteriology. Williams and Williams company, Baltimore. Pp:2431-2508. 1989.
[4] O.H. Lowry., N.J.Rosebrough. and A.L.Fan. Protein measurement

with the folin phenol reagent. J.Biol. chem.. 193: 265-271. 1951.

[5] Mackie and Mc. Cartney. Practical Medical Microbiology. 13th Edition. Chucchill Livingstone, Edinburgh,London, Melbourne and Newyork.2:43-160. 1989.

[6] M. Mandels and J.Weber. The production of cellulases. Advance chem. Ser., 95:391-414. 1969.

[7] T. Okazaki and Y. Okami. Studies on Marine Microorganisms.II Actinomycetes in Sagami By and their antibiotic substances. J. Antibiot. 25:461-466. 1972.

[8] E.B. Sherling and D.Gottlieb, Methods for characterization of Streptomyces species. Int J Syst Bacteriol; 16: 312-40. 1966.

[9] K. Toyota and M.Kimura. Microbial community indigenous to the earthworm Eisenia foetida. Biol.Fertile.Soils. 31: 187-190. 2000.

[10] S.T.Williams., M.Goodfellow., E.M.H. Wellington., J.C. Vickers., G. Aldeson., P.H.A. Sneath et.al. A probability matrix for identification of some streptomycetes. J.of General Microbiology. 129:1815-1830. 1983.