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Original Article

Screening, Optimization and Production of Uricase from a Novel Actinomycete Isolate A85

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ABSTRACT

Background: Uricase is used to diagnose the presence of uric acid in biological fluids as well as to cure gout. Uric acid concentrations in -biological fluids are elevated by several medical disorders. Chronic renal disorders, certain organic acidemias and gout are all possible outcomes of such circumstances. **Objectives:** The current endeavor is primarily focused on isolating new, promising uricase-producing isolate, carrying out molecular identification and optimizing the enzyme's production. **Materials and Methods:** Based on the screening of the isolates for the synthesis of uricase, eight existed isolates in the laboratory were examined for their uricase activity and isolate A85 which showed the maximum zone of clearance was chosen as the promising strain. Based on routine biochemical and physical characterization, it was determined to be a variant of *Streptomyces enissocaesilis* after molecular analysis using the 16S rRNA sequence revealed a 99.73% similarity to *Streptomyces enissocaesilis.* **Results:** Using the One Factor at a Time method in a submerged fermentation process under ideal conditions, *Streptomyces enissocaesilis* produced uricase in concentrations ranging from 0.06 to 3.81 U/mL. The uricase production was increased to 7.53 U/mL from 3.33 U/mL with the optimized medium, which has the following ingredients: pH 9.0±0.2, sucrose (2%), peptone (0.5%), uric acid (0.3%), Na_2HPO_4 (0.1%), NaCl (0.05%), $MgSO_4.7H_2O$ (0.05%) and $CaCl_2$ (0.05%). The isolate A85 nucleotide sequence (16S rRNA) was uploaded in Gene Bank (NCBI) with the Accession number: OR964862.1. **Conclusion:** The investigation showed that the unique pre-existed marine isolate A85 was a potential uricase producer and was identified as a variant of *Streptomyces enissocaesilis*.

Keywords: OFAT Approach, Sucrose, Uric Acid, Uricase, Variant of *Streptomyces enissocaesilis*.

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INTRODUCTION

Urate oxidase, also known as uricase or urate: oxidoreductase (EC 1.7.3.3) is a key enzyme in the purine metabolism that catalyzes the oxidation of uric acid to allantoin.[1] Although most vertebrates contain this enzyme, humans do not.[2] It was first discovered in a cow's kidney. It has also been discovered that uricase can be produced by several natural sources, including bacterial sources such as *Alcaligenes faecalis, Escherichia marmotae, Bacillus subtilis*; fungi sources such as *Aspergillus niger, Gliomastix gueg, Gliocladium viride, Aspergillus welwitschiae* and eukaryotic cells.[3] Uricase produced from various *Streptomyces* sources such as *Streptomyces exfoliate* UR10 and *Streptomyces rochei* NEAE-25.[4,5] The 1st significant use of uricase was in clinical biochemistry as a diagnostic tool for uric acid detection in serum, urin and other biological fluids.[6] Uricase-based methods are best for the determination of uric acid in serum.[7] Compared to other methods, biosensors, that can quickly detect uric acid are more precise, accurate and sensitive.[8] Moreover, uricase can be used as a protein medication to lessen the buildup of poisonous urate in conditions like gout. Higher primates (apes and humans) produce uric acid as a byproduct of purine breakdown because they lack functioning uricase.[9] The inflammatory arthritic disorder known as gout is brought on by the development of monosodium crystals in the tissues and around the joints. Hyperuricemia or elevated serum urate levels, is a medical disorder.[10] Hyperuricemia occurs when serum urate levels rise above 6.8 mg/dL in the blood.[11] Due to its poor solubility, uric acid precipitates in some individuals which can exacerbate gout symptoms.[12] Gout is commonly treated with allopurinol, a potent competitive

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inhibitor of xanthine dehydrogenase, an enzyme that catalyzes the conversion of hypoxanthine to xanthine and xanthine to uric acid.[13] However, urate oxidase can considerably speed up absorption. Chemotherapy-related hyperuricemia problems can be treated or prevented with these injections. These Mammalian creatures were the source of the original uricase.[14] Recently, interest in the production of uricase from microbial sources like preparations fungi, yeast and bacteria has been reported. Since microbial enzymes are inducible, the development of the enzyme requires the existence of uric acid or another inducer in the media. Although several microbiological sources of uricase have been suggested for this therapeutic reason. Pegloticase or Puricase and Rasburicase are produced by r-DNA technology and are FDA-approved drugs.[15] Rasburicase are isolated and refined from *Aspergillus flavus*. It is sold under the trade name uricozyme (Rasburicase) and Puricase is used in the management of treatment-resistant gout and hyperuricemia.[16] Pegloticase is a pegylated, recombinant uricase (urate-oxidase), through vector *Escherichia coli* that exerts its mechanism by the breakdown of uric acid to the water-soluble purine metabolite allantoin.[17] Certain bacteria can synthesize uricase that is controlled by growth media elements and these microorganisms have the inducible ability to break down uric acid and use it for growth.[18] Moreover, it was proposed that a metabolite produced from both the nitrogen and carbon sources may participate in a regulation that regulates uricase production.[19] Several researchers investigated the ideal temperature and pH for bacteria to produce uricase.[20] Numerous writers have looked into how different carbon, nitrogen and other factors affect how microorganisms produce uricase through OFAT and other design methods.[3,4] This study's primary goal is to determine whether local *streptomyces strains* are capable of producing uricase to choose the best isolate and to maximize the uricase production. Based on many literature reviews, most of the uricase reported from microbial sources was intracellular.[21] Extraction and isolation of intracellular enzymes can indeed be challenging due to various factors such as cellular disruption, enzyme stability, purification techniques and optimization of production conditions. The present study specifically investigates the isolation and production of extracellular uricase from a potential source of actinomycetes.

MATERIALS AND METHODS

Preliminary screening of isolates for uricase activity

On a screening medium made up of glucose (0.1%), yeast extract (0.1%), uric acid (0.5%) and agar (2%) with a pH of 7±0.2, the 1st eight isolates A85, A7, A12, A25, A46, A47, A59, A60 were examined. Pure actinomycete isolates were conventionally spot inoculated on a screening medium and incubated at 30ºC for 7 days to perform preliminary screening for the production of uricase. Clear zones that formed around the colonies are evidence of uricase synthesis by the actinomycete isolates.[21] Secondary screening was performed on the isolates that displayed potential uricase activity.[5]

Secondary Screening

The ability to generate uricase was further evaluated using submerged fermentation using a standard medium on the selected isolate A85. After a 7-day incubation period at a temperature of 27ºC, the contents of the flask were subjected to centrifugation at a temperature of 4ºC at a speed of 4000 rpm for duration of 30 min. This process was carried out to obtain a clear supernatant, which was then analyzed for the presence of uricase by conducting uricase assay.[22,23]

Screening of effective uricase production medium

Initially, four different mediums such as PMI consists of Sucrose (2%), Uric acid (0.3%), NaCl (0.05%), K_2HPO_4 (0.1%), $MgSO_4.7H_2O$(0.05%), $FeSO_4.7H_2O$ (0.001%), pH 6.8 and mixed with 100 mL of distilled water**;** PM II-Uric acid (0.3%); K_2HPO_4 (0.2%); KH_2PO_4 (0.05%); $MgSO_4.7H_2O$ (0.01%); NaCl (0.01%); $CaCl_2$ (0.01%); p^H 7.5 and PMIII- Lactose(0.1%), Beef extract (0.1%), uric acid (0.225%), K_2HPO_4 (0.25%), NH_4NO_3 (1.7%) $MgSO_4.7H_2O$ (0.25%), NaCl (0.0002%); $FeSO_4.7H_2O$ (0.0002%); pH 8.8±0.2 and PM IV-Peptone (1%); Sucrose (2%); KH_2PO_4 (0.1%); $MgSO_4.7H_2O$ (0.05%); NaCl (0.05%); $FeSO_4.7H_2O$ (0.001%); Uric acid (0.15%); pH 7.0 were screened for uricase activity.[24,25] For each medium, two flasks (a control flask and a test flask) were constructed. While maintaining the control flasks without adding inoculum A85, test flasks were inoculated with culture A85. All were incubated for 5 days at 28ºC and the fermented flasks were centrifuged at 4ºC for 30 min at a speed of 4000 rpm. The amount of uricase produced during fermentation was determined by uricase assay, either by uric acid plate assay using the acquired clear supernatant.[22]

Measurement of uricase activity by enzyme assay

Both the production of hydrogen peroxide and the depletion of uric acid can be used to determine the enzyme activity of uricase. The depletion of uric acid was measured at 293 nm. The test reaction mixture contains 3 mL of 20 mM sodium borate buffer of pH 9.0 containing 50 µL of 3.57 mM uric acid solution. The test reaction was started by adding 50 µL of crude enzyme (culture supernatant) to the reaction mixture and incubated at 25ºC for 10 min. The blank reaction mixture was prepared the same as the test but instead of 50 µL of crude enzyme, 50 µL of 20 mM sodium borate buffer of pH 9.0 was added. The absorbance was measured at 293 nm by using UV-Spectrophotometer. The difference between the absorbance of the test and blank samples is used to determine the reduction in uric acid concentration. 1 unit (U) of enzyme activity was defined as the amount of uricase needed to convert 1 µM of uric acid per minute at 25ºC and pH 9.0, when taking into the account millimolar extinction coefficient of uric acid (έ) at 293 nm as 12.6 $mM^{-1}cm^{-1}$. The amount of uricase produced was calculated using the formula below.[22]

Uricase calculation formula:

$$Units/mL\ enzyme = \frac{(\Delta A_{293nm/min} Test - \Delta A_{293nm/min} Blank)(B)(df)}{12.6 \times C}$$

Where, ΔA-Absorbance, B-Total volume of reaction mixture, df-Dilution factor, C-Volume of enzyme and 12.6 $mM^{-1} \cdot cm^{-1}$-Molar extinction coefficient of uric acid at 293 nm. The protein content of the uricase enzyme produced by the isolate A85 was estimated to use the Lowry method by taking Bovine Serum Albumin (BSA) as standard.[23-25]

Taxonomical characteristics of selected promising isolate

Morphological, physiological, biochemical and molecular characterization were done to the promising actinomycete strain.[26,27]

Morphological characterization of isolate

During the microscopic analysis the type of mycelium, colour and spore arrangement were taken into account.[19]

Biochemical characterization of isolate

The following biochemical tests were conducted for A85 strain Casein hydrolysis, starch hydrolysis, gelatin hydrolysis, nitrate reduction, methyl red test and indole tests.[28]

Physicochemical characterization of isolate

The isolate's growth and physiological characteristics including pH, temperature and capacity to use various nutrition sources were investigated.[28-30]

Molecular characterization of isolate

The 16S rRNA gene sequencing was used to identify the isolated strains of screening actinomycete. The sequencing was done at NCIM, Pune, India.

Phylogenetic study of the isolate

Evolutionary process was ascertained by using neighbor joining method.[31,32]

Optimization of selected medium

The selected PMI medium is used to maximize the production of uricase and various physicochemical and nutritional are to be optimized. The procedure adopted for the optimization of various parameters influencing uricase production was carried out one after another in a sequential manner (in such a way that the result obtained from the first parameter used in the optimization of second parameter). Before going for optimization, the primary problem associated with uric acid solubility is solved by enhancing the pH of the PMI from 6.8 to 8.5. As this pH is not affected by the growth of the A-85 we maintained this pH till the effect of pH on uricase production is studied.

Evaluation of different fermentation factors for uricase production using One-Factor at a Time Approach (OFAT)

An enhanced uricase enzyme production was obtained through one factor at a time method in consideration with several factors like carbon source, nitrogen source, phosphate source and trace elements, incubation time, pH has a significant role in the production of uricase enzyme by the *Streptomyces* each experiment was performed in triplicate.[25,26]

Effect of carbon source

The effectiveness of various carbon and nitrogen sources was evaluated to assess their impact on the production of the uricase enzyme.[33] Different carbon sources, such as glycerol, dextrose, sucrose, mannitol, CMC (Carboxy Methyl Cellulose) and starch are employed at a concentration of 2% (w/v) in conjunction with uric acid as inducer at a concentration of 0.3% (w/v) investigate their impact on uricase synthesis.

Effect of Nitrogen Source

After analyzing the data acquired from prior trials, an attempt was made to incorporate a nitrogen source at a concentration of 0.5% (w/v) in conjunction with uric acid at a concentration of 0.3%. The subsequent effects of this addition were then examined and evaluated. All nitrogen sources included in the study consisted only of an organic nature, namely peptone, malt extract, beef extract, casein, gelatin and tryptophan, each at a concentration of 0.5%.

Effect of trace salts

Various trace salts, including Calcium Chloride ($CaCl_2$), Sodium Chloride (NaCl), Zinc Sulfate ($ZnSO_4$), Copper Sulfate ($CuSO_4$), were employed as substitutes for Ferrous Sulfate ($FeSO_4$) and Magnesium Sulfate ($MgSO_4$), as 0.05% in the PMI experiment, in conjunction with uric acid at a concentration of 0.3% to investigate the impact of these individual elements.

Effect of pH

The impact of the initial pH of the medium on uricase production by isolating A85 during submerged fermentation was investigated by adjusting the pH of the medium using 4M NaOH and 0.1M HCl. A total of five flasks were prepared, each with a distinct beginning pH value ranging from 7 to 9.5, with increments of 0.5 after incubation for 7 days uricase assay was done for the samples collected from each flask respectively.

Effect of the incubation period

The objective of this experiment was to ascertain the optimal duration of incubation for the A85 isolates when cultured on the

PMI medium. The medium underwent sterilization, inoculation and subsequent incubation. Samples were taken at certain time intervals of 24, 48, 72, 96, 120, 144 and 168 hr. The samples were collected and analyzed after every time interval to determine their uricase activity.[22]

Centrifugation

All the samples were collected in 1.5mL eppendorf tubes after incubation at 27ºC and they were centrifuged at 4000 rpm for 30 min at 4ºC. Then these samples were assayed by using a UV-spectrophotometer.

Production uricase from selected optimized medium obtained through OFAT

The selected optimized production medium I was subjected to submerged fermentation for the production of uricase enzyme, which consists of sucrose (2%), peptone (0.5%), uric acid (0.3%), Na_2HPO_4 (0.1%), NaCl (0.05%), $MgSO_4.7H_2O$ (0.05%) and $CaCl_2$ (0.05%) under optimal conditions, including a pH of 9.0, a temperature of 28ºC and an incubation period of 5 days. The medium weighed 45 mL was inoculated with 5 mL of seed medium under aseptic conditions in a 250 mL conical flask adjusted the pH and sterilized in the autoclave at 120° C,15 lbs. pressure for a duration of 20 min. The experiments were conducted in triplicates and the uricase activity was measured for the supernatant broth according to Mahler *et al*., (1955)[22] and Pustake *et al*., (2019).[33]

RESULTS AND DISCUSSION

Preliminary screening of A85 isolate

Eight actinomycetes existing isolates - A85, A7, A12, A25, A46, A47, A59 and A60 were taken from the existing culture collection of Pharmaceutical Biotechnology, Andhra University, Visakhapatnam previously isolated from marine samples. These isolates were subjected to the uric acid plate assay method. Out of all the isolates, A85 has exhibited the most extensive zone of clearance when subjected to spot inoculation on screening medium. The test reveals the zone of clearance of different isolates. So, isolate A85 was selected for further study.

Selection of seed medium

The preserved cultures were initially revived to growth in starch casein broth. As the cultures are refrigerated standard growth was observed between 1-3 days at 28ºC. After examining the several media for growth of the actinomycete, starch casein broth was selected and used.

Secondary screening

After submerged fermentation for 7 days using the isolate A85 has actively produced uricase. The maximum amount of uricase was produced on the 5th day. Among four different media PMI produced highest uricase activity 3.33±0.14 U/mL, PM-II-2.10±0.05 U/mL, PM-III 1.21±0.07 U/mL and PM-IV 0.68±0.17 U/mL respectively. PMI is further subjected to OFAT optimization.

Microscopy of A85

The isolate A85 was observed under a scanning electron microscope at a required magnification of 6000X as per the standard SEM of the strains and under trinocular microscope it revealed that the spore's surface was smooth and the aerial mass colour was grayish white and possessed chain like structure which is seen in Figure 1 (A). The Sporophore morphology was found to be Mono verticillate with spirals belonging to the *Streptomyces* genera and *Streptomycetaceae* family (spore chain with coiling and branching). This morphology is shown in Figure 1(B).

Morphological examination of isolate A85 clearly indicated that isolate A85 belong to the *Streptomyces* genera and *Streptomycetaceae* family (spore chain with coiling and branching).

Molecular, Phylogenetic and Biochemical of isolate A85

Molecular characterization and Phylogenetic characterization of isolate A85 disclosed that isolate A85 shared proposition of 99.66%, 99.73%, 99.73%, 99.60%, 99.60% sequence similarity with *Streptomyces rochei, Streptomyces enissocaesilis, Streptomyces plicatus, Streptomyces minutiscleroticus, Streptomyces geysiriensis* respectively. Further comparing physiological and biochemical characteristics among isolate A85 with that of closely related isolates (Figure 2, Table 1) indicated that isolate A85 was a variant of *Streptomyces enissocaesilis* as most of its characteristics were similar to A85 except methyl red test and indole test showed negative. When comparing isolate A85 with other isolates it revealed that the major carbon source of isolate A85 was sucrose but isolates such as *Streptomyces rochei, Streptomyces plicatus, Streptomyces minutiscleroticus* and *Streptomyces geysiriensis* not utilizes sucrose as its carbon source whereas *Streptomyces enissocaesilis* utilizes sucrose as its carbon source.[5,34-36] *Streptomyces plicatus, Streptomyces geysiriensis* were not hydrolyzing the casein whereas isolate A85, *Streptomyces enissocaesilis, Streptomyces rochei, Streptomyces minutiscleroticus* hydrolyze the casein. Among all above mentioned stains uricase production was reported only from *Streptomyces rochei* (EI-Naggar 2015).[5] The other species of *Streptomyces* which produce uricase are *Streptomyces exfoliate* (Aly *et al*., 2013),[4] *Streptomyces graminofaciens* (Azab *et al*., 2005),[37] *Streptomyces albosrieolus* (Ammar *et al*., 1987),[38] *Streptomyces aureofaciens* (Demnerova *et al*.,1986)[39] and *Streptomyces cyanogens* (Ohe and Wantanabe,1981).[40] *Streptomyces enissocaesilis* has been previously reported for its L-asparaginase activity (Sirisha *et al*., 2014),[41] algicidal activity (Butsat *et al*., 2023),[42] antibacterial activity (Kumaran *et al*., 2020),[43] and larvicidal and ovicidal activity (Ganesan *et al.*, 2018).[44] We are now reporting its uricase activity for the first time. The neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain A85 and related species of the genus *Streptomyces* (Figure 3).

The isolate A85 nucleotide sequence (16S rRNA) was uploaded in Gene Bank (NCBI) with the Accession number: OR964862.1. As a result, *Streptomyces enissocaesilis* strain A85 was designated as novel variation, based on the isolate A85.

Effect of carbon source

In comparison to other carbon sources, sucrose exhibited a maximum uricase activity of 3.53±0.01 U/mL, while CMC and Mannitol had uricase activities of 0.21±0.01 U/mL each. These values plotted in Figure 4 (a). These findings were obtained using

Table 1: Comparative study of physiological and biochemical characteristics of the isolates A85 with closely related species.

Character	*S. rochei*	*S. enissocaesilis*	*S. plicatus*	*S. minutiscleroticus*	*S. geysiriensis*	Isolate A85
Carbon source:						
Glucose	+	+	++	+	+	+++
Arabinose	+	+	++	+	-	+
Sucrose	-	+	-	-	-	+
Xylose	+	-	++	-	+	-
Fructose	+	+	++	-	-	NA
Starch	NA	NA	NA	+	NA	+++
Cellulose	+	NA	+	-	+	+
Lactose	NA	NA	NA	NA	-	++
Mannitol	NA	+	++	+	+	+
Nitrogen source:						
Tryptone	NA	NA	NA	NA	NA	+
Meat extract	NA	NA	NA	NA	NA	+
Beef extract	NA	NA	NA	NA	NA	++
Malt extract	NA	NA	NA	NA	NA	+
Yeast extract	+	+	+	+	NA	++
Peptone	NA	NA	NA	NA	NA	+++
Degradation of:						
Casein	+	+	-	+	-	+
Starch	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+
Biochemical tests:						
Methyl red	NA	-	NA	+	+	+
Indole	NA	-	NA	+	-	+
Voges-proskauver	NA	+	NA	+	-	-
Nitrate reduction	+	+	NA	+	+	+
Urease	NA	+	+	-	+	+
Opt. pH	8	8-9	8	7.2	8.0	9.0
Opt. temp	35	28	30-40	30	40	30
Pigment	NO	NO	NO	YES	NA	NO
Uricase	+	NA	NA	NA	NA	+
References	El-Naggar (2015)[5]	Butsat *et al.*, (2023)[42]	Abd-allah (2001)[45]	Akshatha *et al.*, (2022)[46]	Poornima et al., (2020)[47]	Present study

+Fair; ++Moderate; +++: Strong; NA- Not available.

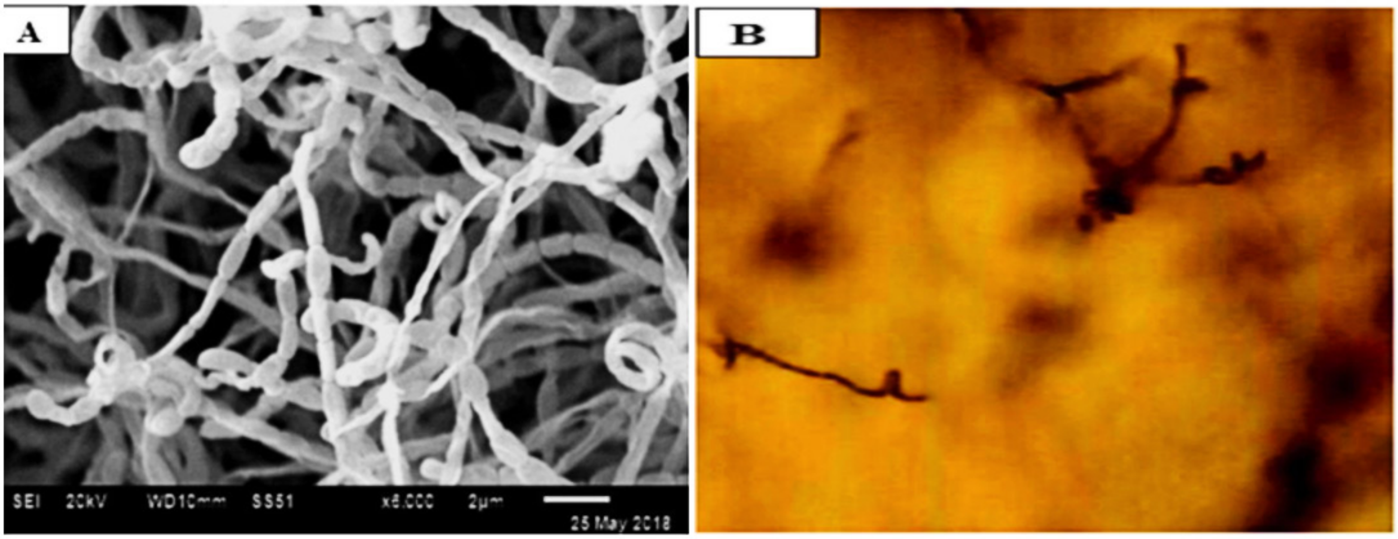

Figure 1: (A): SEM image of isolate A85 (6000X) and (B): Trinocular microscopic image (400X).

isolate A85, which yielded higher uricase activity values than those published by Manimekalai and Anandharaj, B. (2021),[48] for *Bacillus subtilis* MM13. Specifically, the uricase activity for sucrose was reported as 0.69 U/mL in their study. These results agreed with those of Attala *et al*., 2009[49] and Abd El Fattah and Hamed (2002)[50] showed the highest amount of uricase in the medium containing sucrose from *Gliomastix gueg* and *A. flavus* respectively. In a study conducted by Sai Sushma *et al.,* (2023)[51] it was shown that the *Escherichia marmotae* strain (DJDSSS001) had a uricase activity of 25.53 U/mL when maltose is a carbon source.

Effect of Nitrogen Source

Among the several organic nitrogen sources that have been examined, peptone has demonstrated a highly efficient uricase activity of 3.35±0.02 U/mL, while gelatin had uricase activity of 0.52±0.01 U/mL as shown in Figure 4 (b). Peptone was chosen for further evaluation to ensure the optimal concentration necessary for the growth and synthesis of uricase by the isolate A85. According to Pustake *et al*., (2019),[33] it was mentioned that most of the organic sources exhibited a substantial increase in uricase synthesis. Casein showed the highest uricase production (Ali and Ibrahim, 2013).[52]

Effect of trace salts

The production medium containing various trace salts is prepared and sterilized. The samples were obtained from each flask after a 5-day incubation period at a temperature of 28°C. The samples underwent analysis to determine their uricase activity. Out of all the other flasks, the flask containing NaCl exhibited the highest level of activity 3.81±0.01 U/mL, while the lowest activity 1.03±0.01 U/mL was observed at $ZnSO_4$. The results plotted in Figure 4(c). These results agreed with Honarbakhsh *et al*., (2021)[53] reported NaCl at 3% concentration as a significant factor. Aly *et al*., 2013[4] observed that $CaCl_2$ showed the highest activity with *Streptomyces exfoliate* UR10. Uricase can sometimes be found as a tetramer composed of identical subunits, each of which has 2 copper-binding sites. However, this characteristic differs in uricase derived from *Arthrobacter globiformis*. In addition, Cu^{2+}, Fe^{3+} and Zn^{2+} exhibited potent inhibitory effects on uricase activity. However, these ions did not demonstrate any inhibitory effects on Arthrobacter uricase (Suzuki *et al*., 2004).[54]

Effect of pH

The effect of borate buffer at various pH ranges on the growth of isolate A85 was studied. The results of the observation indicate that a pH of 9.0 is the most optimal for the growth of the organism, as it was shown that the highest uricase activity of 3.34±0.02 U/mL, while lowest activity 1.36±0.01 U/mL was recorded at pH 7.0 this particular pH level and the results were plotted in Figure 4 (d). Nanda and Jagadeesh babu, (2014)[55] reported pH 8.5 is optimum for uricase production with *Bacillus cereus*. In another study, *Streptomyces rochei* also showed pH 8 was optimum for the production of uricase reported by El -Nagar 2015.[5] The optimum pH for maximum uricase production was 6.5 reported for *Streptomyces exofoliate* and *Mucor hiemalis* (Aly *et al*., 2013[4] and Yazdi *et al*., 2006).[20]

Effect of the incubation period

Upon studying the effect of the incubation period on uricase production, it was observed that the maximum production of 3.02±0.01 U/mL was observed at 120hrs, while the lowest activity 0.06 ±0.02 U/mL was observed at 24 hr. The values of this study were plotted in Figure 4(e). In another study, *Streptomyces rochei* also showed the highest uricase production on day 5 reported by El-Nagar 2015[5] and Aly *et al*., 2013[4] observed the third day showed the highest activity 3.45 U/mL with *Streptomyces exfoliate* UR10.

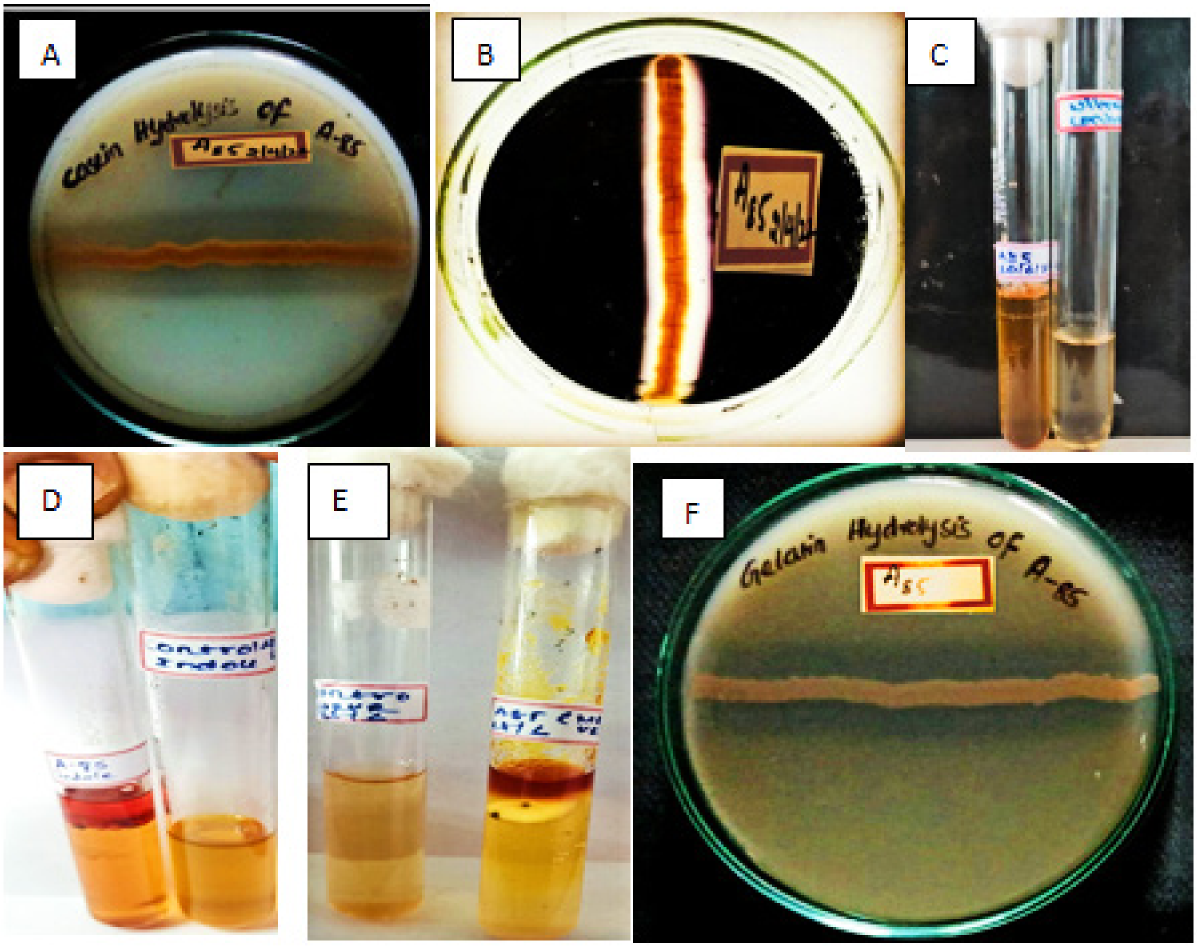

Figure 2: Biochemical characterization of isolate A85. Where A-casein hydrolysis, B-starch hydrolysis, C-nitrate reduction, D-Indole, E-Methyl red test, F-Gelatin hydrolysis.

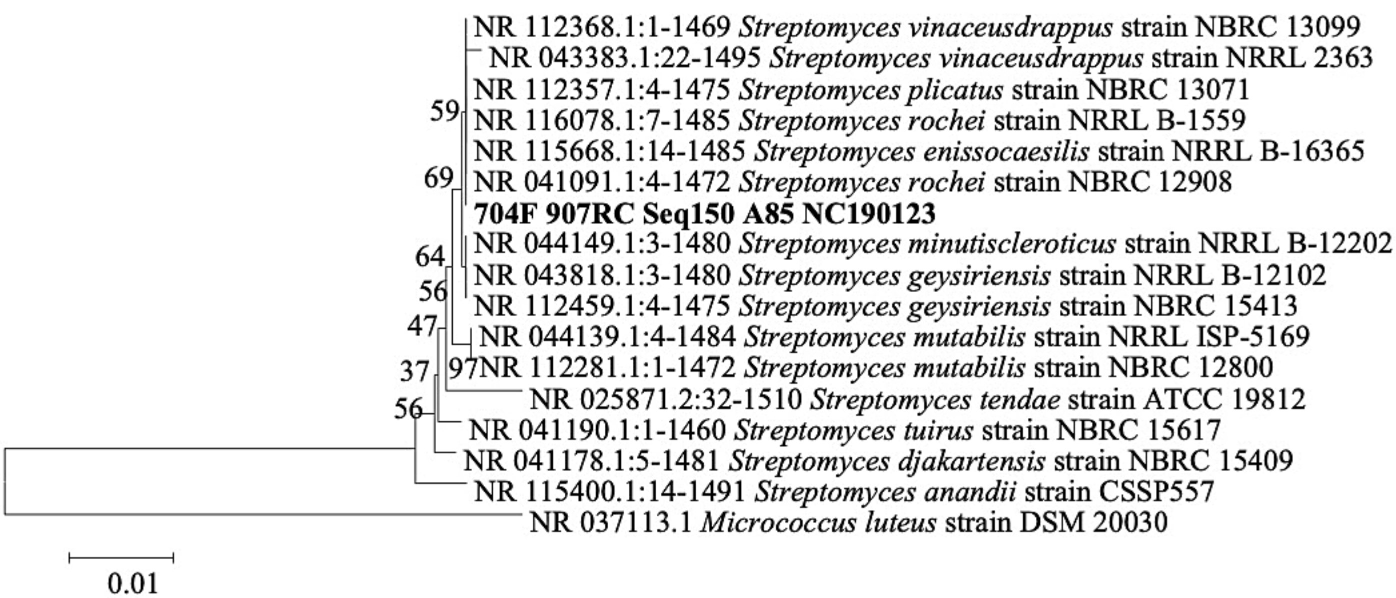

Figure 3: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain A85 and related species of the genus Streptomyces.

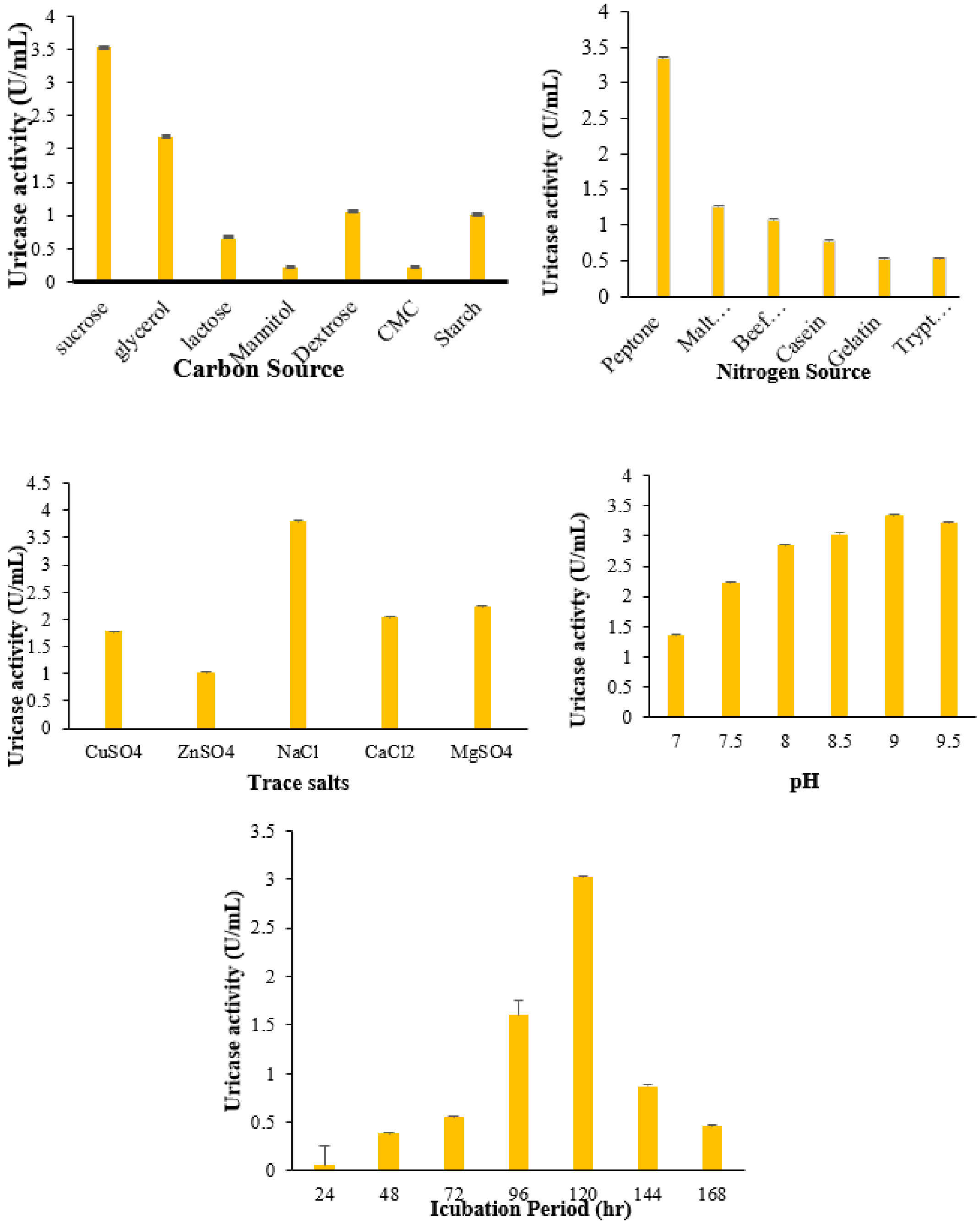

Figure 4: The chart shows the effect of various nutritional source on uricase production by isolate A85; (a) Carbon source (b) Nitrogen source (c) Trace salts (d) pH (e) Incubation period.

Production of uricase from selected optimized medium

The production medium I (PMI) was optimized utilizing the One-Factor-at-A-Time (OFAT) technique and the production of uricase from optimized medium was increased to 7.53 U/mL from an initial level of 3.33 U/mL by using isolate A85. To support this, we performed the optimization studies on uricase production by taking different carbon sources (2%w/v) like sucrose, glycerol, lactose, mannitol, dextrose, CMC and starch. Among all sucrose and glycerol showed the highest uricase activity, nitrogen sources (0.5%w/v) such as peptone, malt extract, beef extract, casein, gelation and tryptophan. Among all peptone showed maximum uricase production. Also, trace salts (0.05% w/v) and NaCl are considered optimum. Varying types of pH have been taken: 7.0, 7.5, 8.0, 8.5, 9.0, 9.5. Maximum production of uricase has been achieved at pH 9, Among varying incubation periods (24, 48, 72, 96, 120, 144, 168 hr) were studied for enzyme production and at 120 hr. maximum uricase production was observed.

The selected optimized production media I which consists of sucrose (2%), peptone (0.5%), uric acid (0.3%), Na_2HPO_4 (0.1%), NaCl (0.05%), $MgSO_4.7H_2O$ (0.05%) and $CaCl_2$ (0.05%) under optimal conditions, including a pH of 9.0, a temperature of 28° C and an incubation period of 5 days. The optimized medium produced 7.53 U/mL of uricase which is higher than reported by Pustake *et al*, 2019[33] produced form *Bacillus subtilis, Streptomyces exfoliates* UR10 produced 0.5U/mL (Aly *et al.*, 2013)[4] and with 0.2 uric acid as inducer, the highest uricase activity of 2.54 U/mL was produced by *B. subtilis* MM13 isolated from pigeon fecal contaminated soil through submerged fermentation process with the chemical components 1% glucose, 0.2% yeast extract and 0.32% of uric acid (Manimekalai and Anandharaj 2021).[41]

In the "one-variable-at-a-time" method, nutritional and physiological parameters were improved progressively. This optimization method is standard and desired. However, this process is time-consuming and does not interact with important nutritional and physiological aspects that could boost biomolecule production. This study also tried the second optimization strategy using Plackett Burman Design (PBD) and a statistical approach. Here, the interactions of the most important production medium parameters for uricase synthesis can be examined and optimized.

CONCLUSION

Isolate A85 is a novel variant of *Streptomyces enissocaesilis* with strong uricase production potential. It represents the first report of uricase activity from *S. enissocaesilis*, expanding its known enzymatic profile. Morphological, biochemical, and molecular characterization confirms its placement in the Streptomyces genus. Optimal medium components and environmental conditions significantly enhanced enzyme yield through OFAT optimizationSucrose and peptone were determined to be the most effective carbon and nitrogen sources for A85 uricase production. NaCl as a trace element played a vital role in enhancing uricase activity, aligning with previous literature. Alkaline pH (9.0) and a 5-day incubation period were optimal for growth and enzyme synthesis. The optimized PMI medium facilitated uricase production more than twice the initial yield, indicating the efficiency of the OFAT approach. The production levels of A85 outperformed several previously reported strains such as *Streptomyces exfoliates, Bacillus subtilis*, and others. The study establishes A85 as a promising strain for potential industrial-scale uricase production and encourages further optimization using statistical methods like Plackett-Burman Design (PBD).

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ABBREVIATIONS

NCIM: National Collection of Industrial Microorganisms; **FDA:** Food and Drug Administration; **16S rRNA:** 16S Ribosomal Ribonuclic acid; **r-DNA:** recombinant DNA; **SEM:** Scanning Electron Microscopy; **OFAT:** One factor at a time method; **PM:** Production Medium; **OD:** Optical Density; **dL:** Deciliter; **cm:** Centimeter; **mg:** Milligram; **nm:** Nanometer; **mL:** Milliliter; **hr:** Hours; **ºC:** Degree Centigrade; **U:** Units; **%:** Percentage; **rpm:** Revolution per minute.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Among eight marine actinomycete isolates, A85 exhibited the highest zone of clearance on uric acid agar, indicating strong uricase activity. Morphological, SEM, biochemical, and 16S rRNA gene analysis identified A85 as a novel variant of *Streptomyces enissocaesilis* with 99.73% sequence similarity. Starch casein broth was selected as the ideal medium for reviving A85 cultures. Secondary Screening relived thatA85 showed maximum uricase production on day 5, with the best results in Production Medium I (PMI) at 3.33±0.14 U/mL. SEM and light microscopy revealed monoverticillate sporophores with spiral spore chains which are characteristics of the *Streptomyces*. Sucrose yielded the highest uricase activity (3.53±0.01 U/mL), confirming its preference as a primary carbon source. Peptone was the most efficient nitrogen source (3.35±0.02 U/mL) for uricase production. Among salts tested, NaCl resulted in the highest uricase activity (3.81±0.01 U/mL), whereas $ZnSO_4$ inhibited production. The optimal pH for uricase production was pH 9.0, and the best incubation period was 120 hr, giving 3.02±0.01 U/mL. Using OFAT optimization, uricase production was enhanced to 7.53 U/mL in optimized PMI medium—significantly higher than previously reported levels.

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