

Production and partial purification of α -amylase producing *Streptomyces* sp. SNAJSM6 isolated from seaweed *Sargassum myriocystum* J. Agardh

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The bacteria *Streptomyces* sp. was isolated from the seaweed *Sargassum myriocystum* that was collected from Kilakarai, Ramanathapuram District, Tamil Nadu. Among the isolates, the SNAJSM6 produced the maximum content of extracellular α -amylase, which was confirmed by starch hydrolysis and identified as *Streptomyces* sp. SNAJSM6. The culture optimization studies for augmented enzyme production in the *Streptomyces* sp. were conducted under different physico-chemical parameters, such as pH, temperature, and carbon and nitrogen sources for α -amylase production. The maximum α -amylase production was recorded on the third day of incubation (56U/ml) by *Streptomyces* sp. SNAJSM6. The *Streptomyces* sp. optimized α -amylase enzyme production was recorded at pH 7.0 and temperature 30 °C, which was 1.86 fold higher than the unoptimized culture broth of the *Streptomyces* sp. The *Streptomyces* sp. α -amylase enzyme was partially purified and its molecular weight was found to be 44 kDa by using SDS-PAGE. Among the isolates that were screened, *Streptomyces* sp. SNAJSM6 is a potential candidate for maximum production of α -amylase. Further, it showed excellent antibacterial activity against selected human pathogens.

[**Keywords:** *Sargassum myriocystum*; *Streptomyces* sp.; α -amylase; Antibacterial activity]

Introduction

Marine environment is one of the richest sources of microorganisms¹. The enzymes that are produced by marine microorganisms are considered to be highly important for medical and industrial purposes. High salinity in the marine environment provides a unique and rare microbial product that could be used for therapeutics². Marine actinomycetes have unique physical and biochemical characteristics and potent bioactive molecules and they release a broad diversity of biologically and pharmaceutically important active enzymes³. Thus, actinomycetes associated with marine seaweeds and animals are expected to be a potential source for new bioactive molecules⁴. Amylases are characterized by their ability to hydrolyze starch and related carbohydrates. They are used in a wide variety of industries, such as clinical, textile, detergent, paper, food, pharmaceutical, and others⁵. Seaweed associated microbes are unique and have not been studied well. Two promising *Streptomyces* sp. have been reported recently for their amylase production⁶. Seaweed can be a host of many epiphytic and heterotrophic bacteria for mutualism of both organisms⁷. For the present study, *Streptomyces* sp. SNAJSM6 were isolated from the seaweed *Sargassum myriocystum*. Industrially important

enzymes, such as amylase, cellulose, and lipase producing marine *Streptomyces* sp. were screened and their production was assessed. Among the isolates, SNAJSM6 produced maximum content of extracellular α -amylase, which was confirmed by starch hydrolysis and identified as *Streptomyces* SNAJSM6. Further, the production of α -amylase was enhanced by production media from *Streptomyces* sp. The α -amylase enzyme was partially purified from *Streptomyces* sp. (44 kDa) and the antibacterial activity was studied against selected human pathogens.

Materials and Methods

Sample collection

The brown seaweed *S. myriocystum* J. Agardh was collected during a low tide in the south-east coastal area of the Kilakarai (9.2343° N, 78.7836° E), Tamil Nadu, India (Figs. 1a–c). The sample (500 g) was kept in a zip-lock cover for further investigations. The algal thallus was rinsed with tap water to remove the debris and epiphytes. Once the sample had been taken, the algal surface was rinsed again with sterile water and stored in a sterile flask. A sterile swab was used to rub the seaweed surface and the removed bacteria were inoculated on marine agar and marine



Fig. 1 — (a) Map view of sample collection site, (b) Seaweed sample collection site (Keelakarai), (c) Seaweed *Sargassum myriocystum* (J. Agardh), and (d) *Streptomyces* sp. isolated strain SNAJSM6

broth isolation plates. Along with agar medium, cyclohexamide (25 $\mu\text{g/ml}$) was supplemented to inhibit the fungal growth. The inoculated plates were incubated for 24–48 h at 30 °C until colonies were observed. Seven colonies were isolated from the marine macroalgae. Bacterial isolates were purified through the third generation for subsequent re-streaking of the bacteria and stored in an agar slant at 4 °C (Fig. 1d).

Biochemical characterization

The actinomycetes isolates were characterized using the following: (i) Amylase production, (ii) methyl red, (iii) Vogus Proskauer, (iv) starch hydrolysis, (v) spore formation, (vi) citrate, and (viii) indole test according to the International *Streptomyces* Project methods. The reaction was stopped by adding 1 ml of DNS and the absorbance was measured at 540 nm^{8,9}.

Optimization of α -amylase production

The production of α -amylase was carried out in a liquid media containing starch (1%), yeast extract (0.5%), peptone (0.5%), K_2HPO_4 (0.1%), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.005%), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.002%), $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.001%), and NaCl (3.0%). In seawater, distilled water (1:1), and artificial seawater (ASW), the pH of the culture medium was adjusted to 7.0. For the optimization of *Streptomyces* sp. SNAJSM6, 5% of inoculums was added to the culture flasks and incubated in a rotary shaker at 28 °C for 36–48 h at 120 rpm. After specific intervals of time, the samples were taken out to determine the enzyme activity and protein partial purification by ammonium sulfate precipitation. The molecular weight was determined by SDS-PAGE. The physical and chemical parameters of fermentation conditions, such as agitation, pH, temperature, and carbon and nitrogen

sources, were studied to identify the suitable condition for the maximum production of α -amylase¹⁰.

Estimation of protein

The protein content at different stages of purification was estimated by utilizing the principle of protein-dye binding¹¹ using bovine serum albumin (BSA) as the standard. To 10–100 μ g of protein and suitable aliquots of the sample in 1 ml, 5 ml of alkaline copper reagent (50 ml of 2% NaCO₃ in 0.1 N NaOH mixed with 1 ml of 0.5 % CuSO₄ in 1% sodium potassium tartrate) was added and mixed thoroughly. After 10 min of incubation at room temperature, 0.5 ml of 1 N Folin's reagent was added and the mixture was vortexed. A blue colour developed after 30 min of incubation in the dark and the optical density (OD) was measured against the blank at 660 nm using Hitachi U2900 UV-visible spectrophotometer.

Partial purification of α -amylase

Streptomyces sp. SMAJSM6 was cultured for 24–48 h in an optimized starch broth at 30 °C. After incubation, the culture was centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was collected and the pellet containing the cell debris was discarded. Ammonium sulfate was added to the culture supernatant to get 0–60% saturation level from the initial zero concentration. The precipitated solution was then centrifuged at 10,000 rpm for 15 min at 4 °C. The pellet obtained after centrifugation was dissolved in a minimum volume of 0.2 M phosphate buffer (pH 7.0) and overnight dialysis was performed using 10,000 MW cutoff dialysis bag for the precipitate against 0.2 M phosphate buffer with respective changes of phosphate buffer. Then it was centrifuged at 10,000 rpm at 4 °C and the pellet was separated and freeze-dried.

Determination of molecular weight of α -amylase by SDS-PAGE

SDS-PAGE of α -amylase was performed using the standard method¹². The experiment was conducted using 10% separating gel and 5% stacking gel under non-reducing. The standard protein molecular weight marker was run along with the enzyme samples. Electrophoresis was stopped when the tracking dye (bromophenol blue) reached the bottom of the gel. The proteins in the gel were stained with Coomassie brilliant blue R-250 and destained with a solution containing 5% methanol and 7% acetic acid.

Antibacterial activity

Streptomyces sp. α -amylase was tested for antibacterial activity using well-diffusion method¹³. The extracted active molecule was tested against human pathogens, such as *Pseudomonas aeruginosa*, *Bacillus cereus*, *Enterobacter* sp., *Salmonella* sp., and *Micrococcus luteus*. For comparison, the antibacterial ampicillin was used as the standard antibiotic. The media used were Mueller Hint on agar for bacteria. The plates (duplicates) were incubated at 37 °C for 18 h in the case of bacteria. The antibacterial activities of α -amylase were determined by measuring the respective zones (mm) of inhibition.

Results and Discussion

Screening of microorganisms

In the present study, the brown alga *S. myriocystum* associated seven bacteria and actinomycetes, namely, SNAJSM1, SNAJSM2, SNAJSM3, SNAJSM4, SNAJSM5, SNAJSM6, and SNAJSM7, were isolated and identified morphologically and microscopically (Figs. 1c and d). The study of starch hydrolysis by α -amylase was carried out using the agar plate technique, and the enzyme activity was observed only on the isolate of SNAJSM6 to produce a clear zone around the colonies. Screening of amylase, cellulase, caseinase, chitinase, gelatinase, and lipase enzyme producing *Streptomyces* sp. was streaked on the respective plates (Fig. 2). The isolate shows that different physico-chemical parameters are influencing the growth rate of *Streptomyces* sp. SNAJSM6. Earlier studies on *S. carpaticus* have shown that active strains grow in 25 °C and pH 7.0^{14–16}. In the present study, different biochemical tests were carried out and the results are shown in Table 1. The α -amylase producing organism *Streptomyces* sp. SNAJSM6 is shown in Figures 2a-f. *Streptomyces* sp. SNAJSM6 isolate was prominent among the seven bacterial isolates that exhibited hydrolysis zones of

Table 1 — Biochemical test for α -amylase Producing *Streptomyces* sp. SNAJSM6

Biochemical test	Results
Grams stain	Positive
Indole test	Positive
MR test	Positive
VP test	Positive
Catalase test	Positive
Citrate utilization	Negative
Urease test	Positive
Starch hydrolysis	Positive
Casein hydrolysis	Positive

α -amylase. Based on the production of α -amylase, *Streptomyces* SNAJSM6 was chosen for further studies. Recently, isolated *Streptomyces* genus from aquatic sediments have been reported for the production of amylase and screening of marine actinomycetes for production of enzymes^{17,18}. When maltose and peptone were used as substrate¹⁹, high amylase activity (150 U/mL) was detected by *Penicillium fellutanum*, after four fermentation days.

In *Streptomyces* sp. D1, the maximum amylase production (4.5U/mL) was observed 10 days after fermentation using sucrose and malt extract²⁰. We used similar substrate, and starch and yeast extract recorded the maximum amylase activity of 55 and 54 U/ml, respectively (Figs. 3d and e). Maximum enzyme activities (54 U/mL) of *Streptomyces* sp. SNAJSM6 isolate have been recorded in this study at even 40–70 °C (Fig. 3b). A previous study from

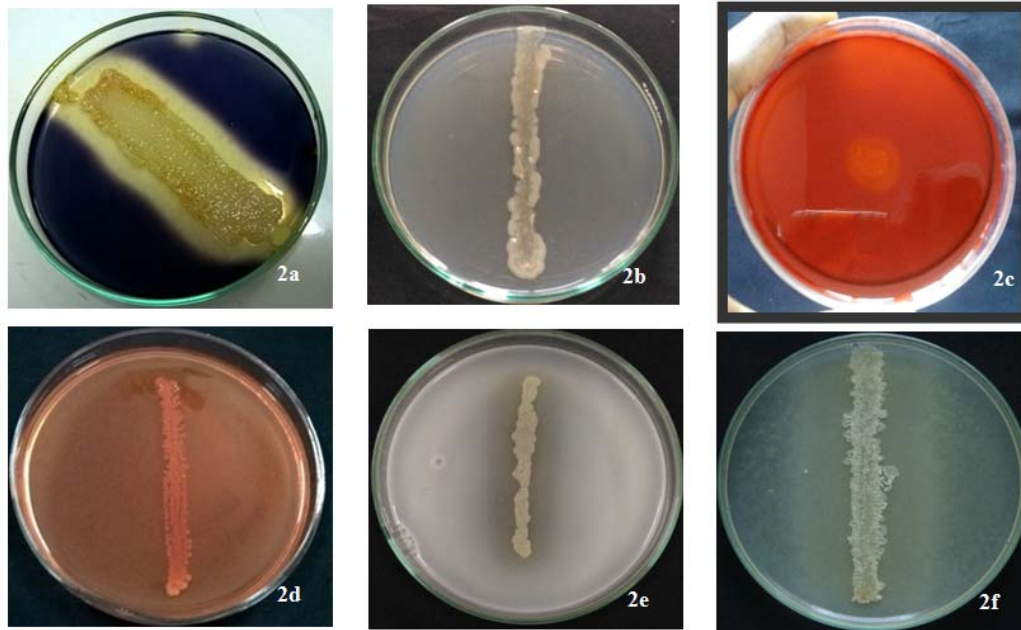


Fig. 2 — Different enzyme production assay for *Streptomyces* sp: (2a) Amylase, (2b) Caseinase, (2c) Cellulase, (2d) Chitinase, (2e) Geletinase, and (2f) Lipase

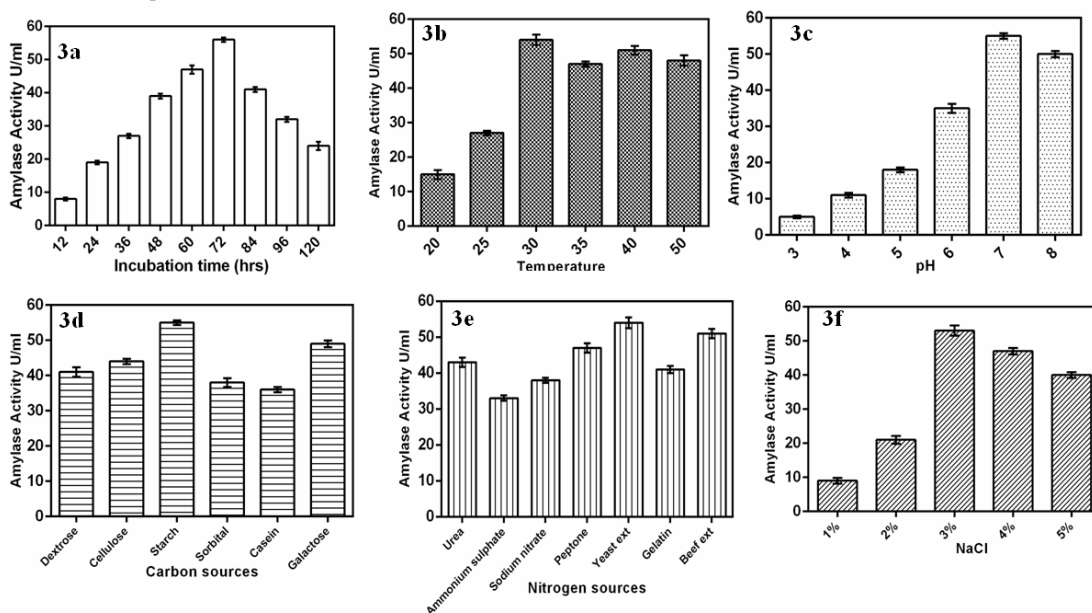


Fig. 3 — Different optimization conditions of α -amylase producing *Streptomyces* sp.: (3a) Different incubation (hrs), (3b) Different temperature, (3c) Different pH, (3d) Different carbon sources, (3e) Different nitrogen sources, and (3f) Different salinity

marine *Streptomyces* sp. D1 produced novel α -amylase enzyme at 80 °C²⁰, and also a thermostable α -amylase from moderately thermophilic *B. subtilis* strain for starch processing²¹. The pH activity profiles of *Streptomyces*, within the range between 3 and 9, indicated that the maximum activity was observed (55 U/mL) at pH 7.0 and also significant levels of activity were observed between pH 5.0 and 8.0 (Fig. 3c). The biocatalyst was able to perform in a broad range of pH, but the neutral range pH produced good results in *Aspergillus awamori*²², and also a *Bacillus* strain had maximum amylase activity in pH 9.0, and the range 7.0–11.0 showed good performance²⁰. The seaweed associated microorganism *Streptomyces* sp. SNAJSM6 used in this study was able to produce α -amylase using starch and yeast as carbon and nitrogen sources. At different incubation periods, the maximum α -amylase activity detected was 56 U/mL on the third day of incubation (Fig. 3a), when the salt mineral medium was supplemented with starch (1%) and yeast (0.5%), which was 1.86 fold higher than the unoptimized culture broth of the enzyme produced from *Streptomyces* sp. However, the *Streptomyces* sp. SNAJSM6 isolate had better activity in 3% NaCl concentration (Fig. 3f). Among the seven isolates, the *Streptomyces* sp. SNAJSM6 had potent antibacterial activity against selected human pathogens as shown in Figure 4. The α -amylase obtained from *Streptomyces* sp. SNAJSM6 showed good antibacterial activity against the following five selected human pathogens: (i) *P. aeruginosa*, (ii) *B. cereus*, (iii) *Enterobacter* sp., (iv) *Salmonella* sp., and (v) *M. luteus*. The isolate SNAJSM6 showed higher zone of inhibition in 21, 17, and 16 mm of *Enterobacter* sp., *Salmonella* sp., and *P. aeruginosa*, respectively. The minimum zone of inhibition was recorded in 15 and 12 mm of *B. cereus* and *M. luteus*, respectively. Earlier publications showed similar results in the epiphytic marine bacteria associated with seaweeds with a zone of inhibition of 10 mm against *Staphylococcus aureus*, *Escherichia coli*, and *Alcaligenes faecalis*^{23,26}. Likewise, 13 promising marine bacteria isolates from seaweed, seawater, and sediment, from that *Lysinibacillus xylanilyticus* GT134 showed potent antibacterial activity against plant pathogen *Xanthomonas oryzae* and *X. anopodis*²⁷. The *Streptomyces* sp. produced α -amylase enzyme was partially purified and molecular weight was found to be 44 kDa by using SDS-PAGE (Fig. 5). The *Streptomyces* sp. SNAJSM6 produced a partially

purified bioactive α -amylase enzyme of molecular weight 44 kDa, which showed excellent antibacterial activity against selected human pathogens²⁸. A previous report states that the haloalkaliphilic marine *Saccharopolyspora* sp. produced a low molecular weight bioactive molecule, α -amylase enzyme of 66 kDa, by using SDS-PAGE, in the ammonium sulfate precipitation method²⁰.

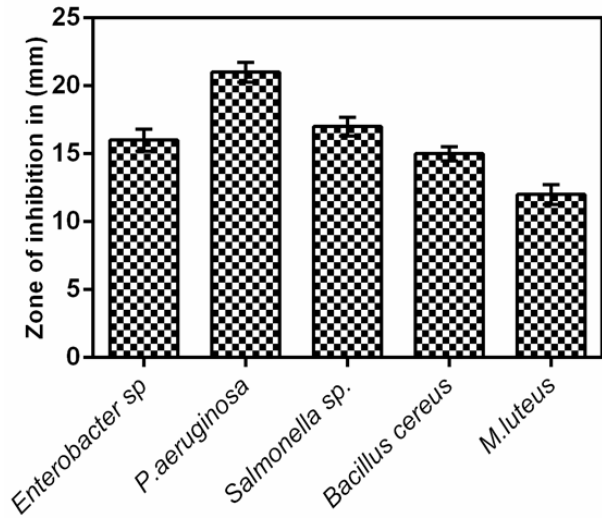


Fig. 4 — Antibacterial activity of partially purified α -amylase producing *Streptomyces* sp. isolated from *Sargassum myriocystum* against human pathogens

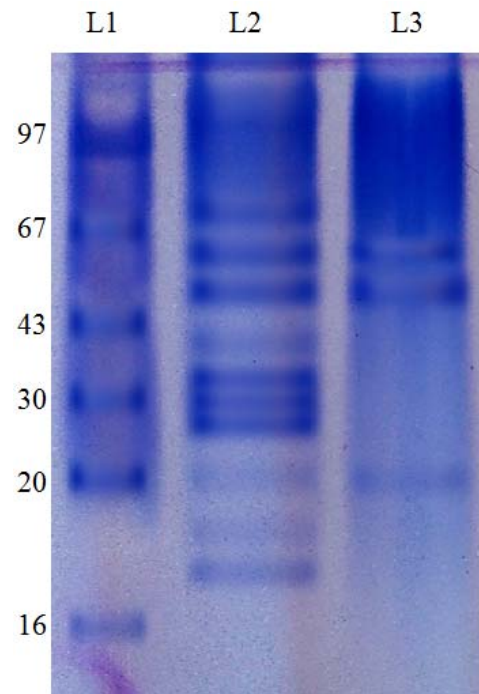


Fig. 5 — Partial purification of α -amylase producing *Streptomyces* sp. Lane 1, ladder; Lane 2, crude; Lane 3, ammonium sulfate precipitation (30–60%)

Conclusion

The present study has revealed that the potent isolate of *Streptomyces* sp. SNAJSM6 isolated from brown alga *S. myriocystum* from the south-east coast of Tamil Nadu, India, could be used for the α -amylase production. The α -amylase production was found maximum in the presence of the following: (i) Starch as a carbon source, (ii) yeast extract as a nitrogen source, (iii) 3.0% of NaCl concentration, (iv) temperature 30°C, and (v) pH 7.0. The enzyme was partially purified and the molecular weight was found to be 44 kDa by using SDS-PAGE. At the optimum conditions, *Streptomyces* sp. SNAJSM6 produced 56 U/mL of bioactive enzyme in the culture broth, which was 1.86 fold higher than in the unoptimized culture broth. This investigation concludes that the bioactive, potent α -amylase enzyme produced from seaweed associated bacterium *Streptomyces* sp. SNAJSM6 could be used as a broad spectrum antibacterial agent. Further, the potential of α -amylase produced by *Streptomyces* sp. SNAJSM6 showed excellent antibacterial activity against selected human pathogenic bacteria.

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