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Research Article

OPTIMIZATION OF ALPHA AMYLASE EXTRACTED FROM MARINE ACTINOMYCETES-*STREPTOMYCES GANCIDICUS_ASD-KT852565*

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ABSTRACT

The crude extract obtained from Streptomyces gancidicus_ASD-KT852565 isolated from marine actinomycetes soil sample which was collected from Andaman & Nicobar Island was subjected to optimization with 10 different parameters like temperature, Nitrogen source, Carbon source etc. for better yield of alpha amylase. The results obtained showed that the enzyme production by S.guancidicus_ASD-KT852565 was optimum at temperature 40°C, pH 7.0, with carbon source as sucrose, Nitrogen source as Beef extract, Amino acid as L-asparagine and also at NaCl concentration of 1.5 %, volume of inoculum as 50ml in 250 ml flask, incubation time maintained for 96 hours enhanced with $MgSo_4$ as metal ion and finally starch as substrate at the concentration of 50g/L showed good yield applicable for industrial use.

Keywords: Andaman & Nicobar island, Marine soil sample, Actinomycetes, Alpha amylase, optimization.

INTRODUCTION

Actinomycetes are filamentous Gram-positive bacteria, characterized by a complex life cycle belonging to the phylum Actinobacteria, which represents one of the largest taxonomic units among the 18 major lineages currently recognized within the Domain Bacteria[1]. The isolation of marine actinomycetes has been a great source of new compounds and their isolation all around the world from deepest sediments to the shallow costal sediments, demonstrates that actinomycetes are ever-present in marine sediments, but at lower numbers than in soil[2,3,4,5]. In the case of marine actinomycetes several attempts to optimize their isolation and growth from several sources[6, 7, 8].

Amylase is the enzyme which hydrolyzes starch. It is used to convert starch to glucose and in paper, textile, alcohol (from starch), nutritional and detergents industries [9, 10]. Nowadays it is applied in medicine, biotechnology and chemistry, too[11]. The most important bacterial genera that produce amylase are Bacillus, streptomyces, Micrococcus, Pseudomonas, Arthrobacter, Escherichia, Proteus and Serratia[12, 13]. Applications of amylases includes, processing of fruits like mango, banana, papaya and citrus fruits[14] ,used in laundry and dish washing detergents[15] .There are various reports on amylase production by actinomycetes. A promising *Streptomyces clavifer* and *Streptomyces* spp.[16] have been reported. Recently[17] isolated *Streptomyces* genus from aquatic sediments for the production of amylase & screening of marine actinomycetes for production of enzymes[18] have been reported.

In order to achieve commercial benefit, it is totally necessary to economize production (greater volumetric productivity within a very short time) in the easiest available way. Enzyme overproduction may be achieved by both genetic manipulations and media engineering. Genetic manipulation methodology is complex and rather expensive. Stability of recombinants is an issue of doubt that sometime less popularizes use of overproducers in industry. As an alternative way, manipulation of physico-chemical conditions that positively influence the production machinery has been considered a better strategy[19].Such optimizations not only allow overproduction but also lead to optimized selection and use of nutrients by minimizing their losses. Currently the classical approach of media optimization has lost its significance, mainly due to its failure of finding interactions between independent variables i.e. physical (temperature, pH etc.) and chemical (media components) factors [20]. Fresh water actinomycetes are one of the important resources for screening useful enzymes and bioactive metabnolites.[21]

MATERIALS AND METHOD

Actinomycetes strain and media

Streptomyces gancidicus_ASD-KT852565 isolated from marine soil sample collected from Andaman & Nicobar Islands producing amylase was used for study. The strain under research was stored at 4°C till the end of the study. The starch agar media containing starch casein agar medium (g/L Starch-10; Casein-0.3; KNO3-2; NaCl-2; K2HPO4; MgSO4.7H20.05; CaCO3-.02;FeSO4.7H2O-0.01; Agar-20), supplemented with antibiotics Cyclohexamide (20 µg/mL) and Nalidixic acid (100µg/mL) to prevent fungal and bacterial growth respectively.

Enzyme activity estimation

Amylase activity was assayed using a reaction mixture comprising of 1 ml of crude enzyme, 1 ml of 1% (w/v) soluble starch solution .The reaction mixture was incubated at 25°C for 10 min. and the reaction was terminated by adding 2 ml of DNS to the reaction was terminated by adding 2 ml of DNS to the reaction tube and then immersing the tube in the boiling water bath (100°C) for 5 minutes. Reducing sugar (maltose) liberated was estimated by the 3, 5 Dinitrosalicylic acid (DNS) method. Absorbance was measured at 246 nm with spectrophotometer. One unit of amylase activity was defined as the

amount of enzyme causing the release of 1µ mole of reducing sugar in one minute, under assay conditions[22].

Protein content estimation

Protein content was assayed using a reaction mixture compromising of 1ml of crude enzyme, 1ml of substrate solution and incubated for 10 minute. Reaction was stopped by 0.1 phenol solution incubated for 30 min at room temperature. The absorbance was measured at 650 nm with UV-Visible spectrophotometer[23].

Effect of Temperature

One of the major influencing factors in optimization is the temperature maintained for the organisms' growth, enzyme activity and its protein content. In the present study 50 ml of the crude enzyme was incubated at varying temperatures ranging from 15, 20, 28, 37, 40, 45 to 50°C.

Effect of pH

pH (biocatalyst) is the most important factor which markedly influences the enzyme activity. In this study pH varied from 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 to 9.0 under maintained temperature.

Effect of Carbon source

On the other hand, Culture broth was distributed into various flasks and 1% of each carbon source viz. sucrose, fructose, Dextrose, Lactose, Glucose, Galactose, Mannose , Trehalose, Melibose, Xylose,was then added before inoculating of the strain and was incubated at the optimum pH, temperature.

Effect of Nitrogen source

Also, Culture broth was used for studying the effect of various nitrogen compounds viz. peptone, yeast extract, Ammonium sulphate, Beef extract, Sodium acetate, Meat extract, Urea, Nutrient Broth, Potassium nitrate, Sodium citrate. The broth was distributed into various flasks and 1% of each nitrogen source was then added before strain inoculation. Cultures were incubated at already standardized parameters.

Effect of Amino-acids

Even though Amino acids are considered under trace minerals it enhances the yield of amylase and this was tested by varying the amino acids like L-glutamine, L-asparagine, L-tyrosine, L-lysine, L-histidine, L-cysteine, L-arginine, L-therionine and L-tryptophan in the culture by maintaining other optimized parameters.

Effect of Medium volume

The volume of medium decides the proper aeration to the culture which is reflected in the growth of the enzyme. The effect of medium volume was studied at different volumes in 250 ml flask like 20ml, 25 ml, 30ml, 35ml, 40ml, 45ml, 50ml, 55ml, 60ml, and 70ml under above optimized condition.

Effect of Incubation time

The Incubation time of the culture decides the maturity of the cell for active enzyme production. Production of amylase is optimized by varying the incubation holding time from 12hrs, 24hrs, 36hrs, 48hrs, 60hrs, 72hrs, 84hrs, 96hrs, 108hrs and 120hrs.

Effect of NaCl concentration

The utilization of the NaCl determines the salinity level of the organism which also determines the nature of the organism (terrestrial or marine). Thus here, the various concentrations of NaCl like 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 were used to find the optimizing level.

Effect of Metal ions

The trace elements which include metal ions also impact on the production of amylase which was studied by varying the metal ions like $CuSo_4$, $MgSo_4$, KNo_3 and $CaCo_3$. In the enzyme action, metallic cofactors are important because their presence or absence regulates enzyme activity. The presence of specific metallic ions along with essential nutrient source can inhibit or enhance amylase activity.

Effect of Substrate concentration

Addition of Substrate and its concentration plays a major role in production of amylase. The effect of starch concentration was studied by varying its concentration such as 10ml, 20ml, 30ml, 40ml, 50ml, 60ml, 70ml, 80ml, and 90ml per litre where all the other parameters where under optimized state.

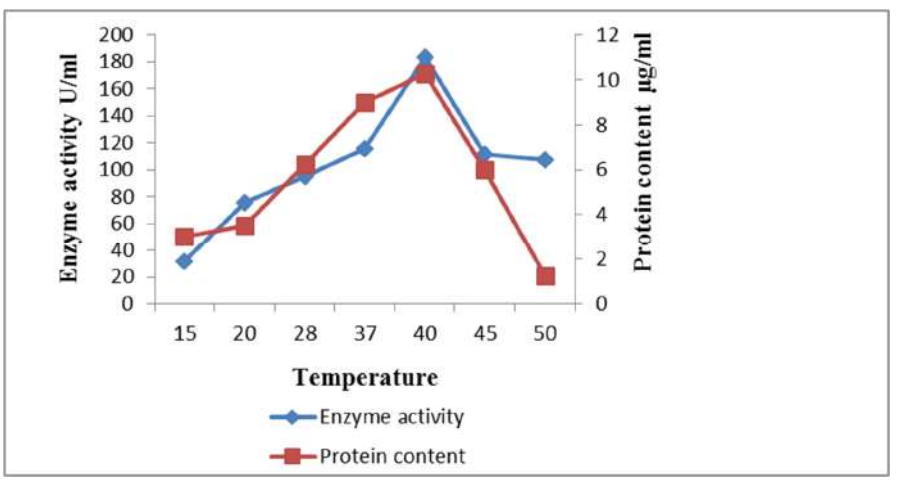

Figure 1: Effect of temperature on Enzyme activity and Protein content

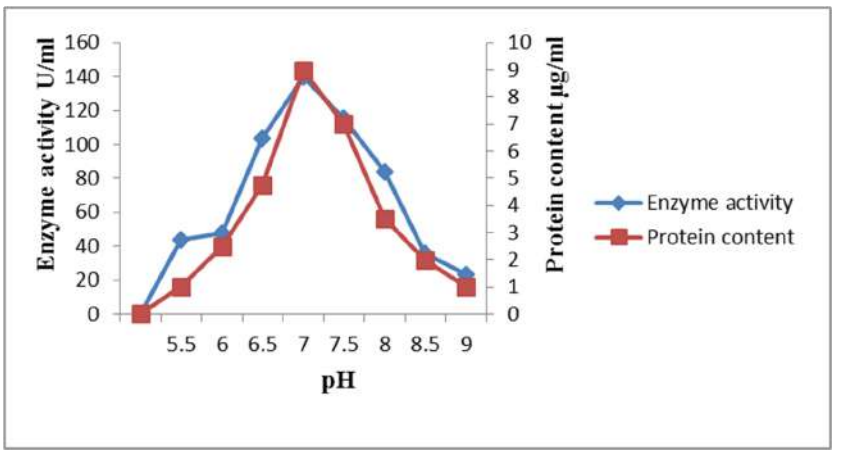

Figure 2: Effect of pH on Enzyme activity and Protein content

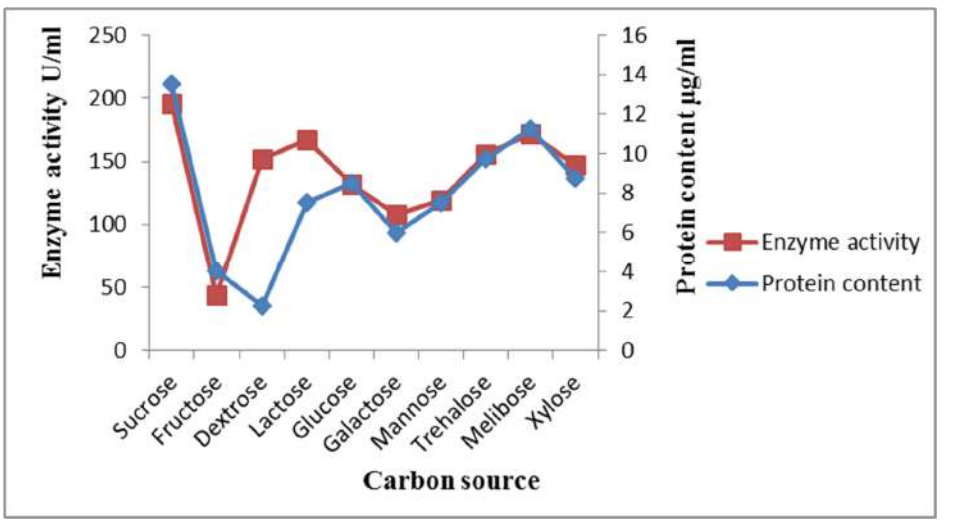

Figure 3: Effect of Carbon source on Enzyme activity and Protein content

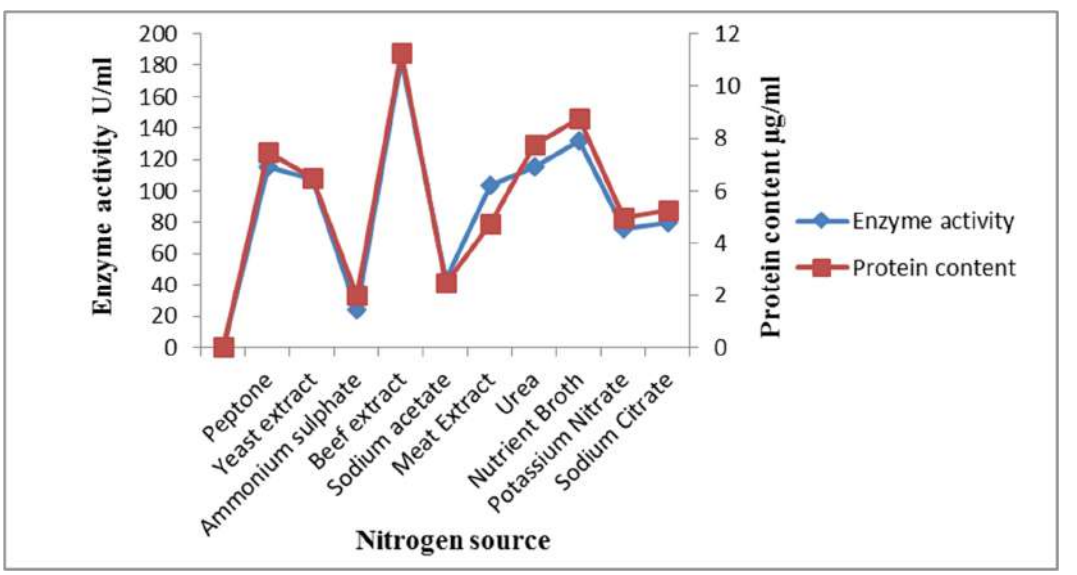

Figure 4: Effect of Nitrogen source on Enzyme activity and protein content

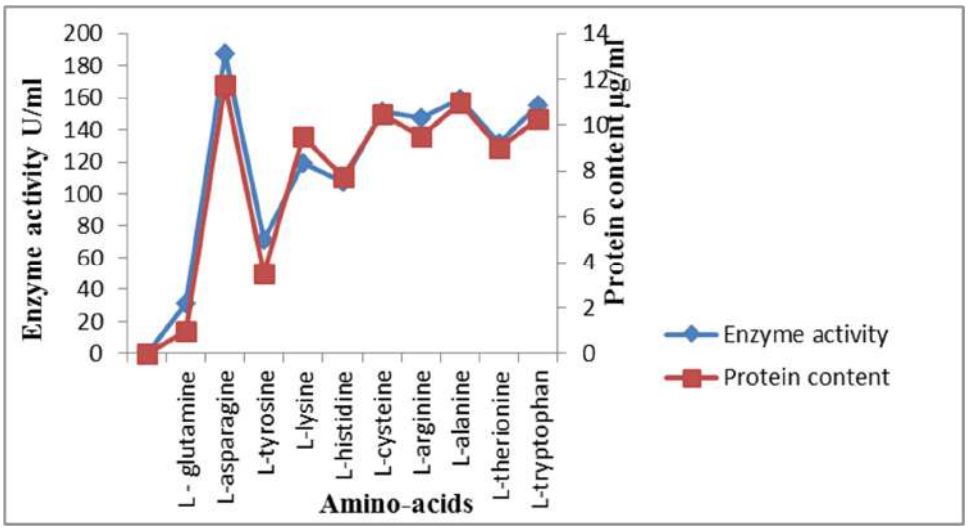

Figure 5: Effect of Amino-acids on Enzyme activity and Protein content

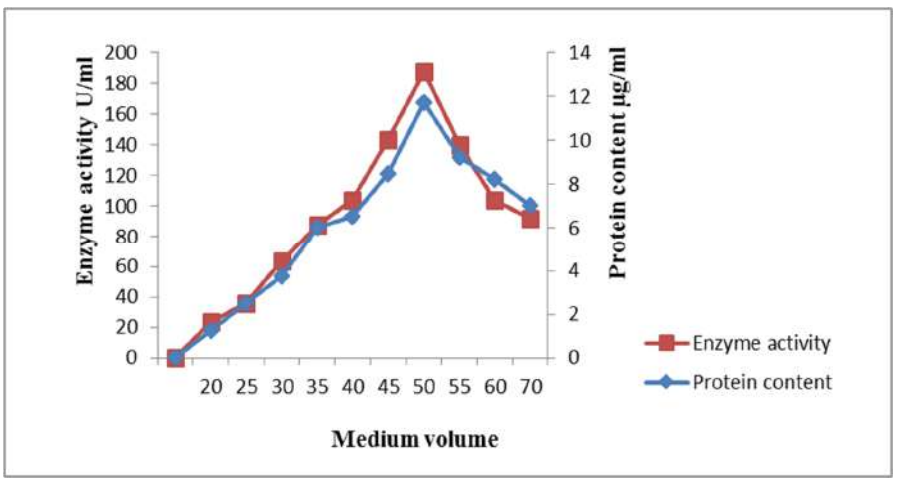

Figure 6: Effect of Medium volume on Enzyme activity and Protein content

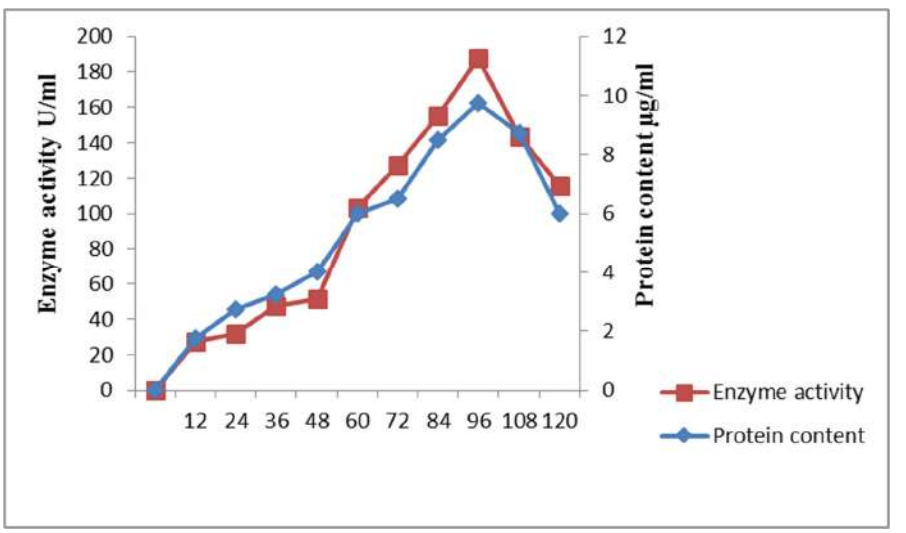

Figure 7: Effect of Incubation time on Enzyme activity and Protein content

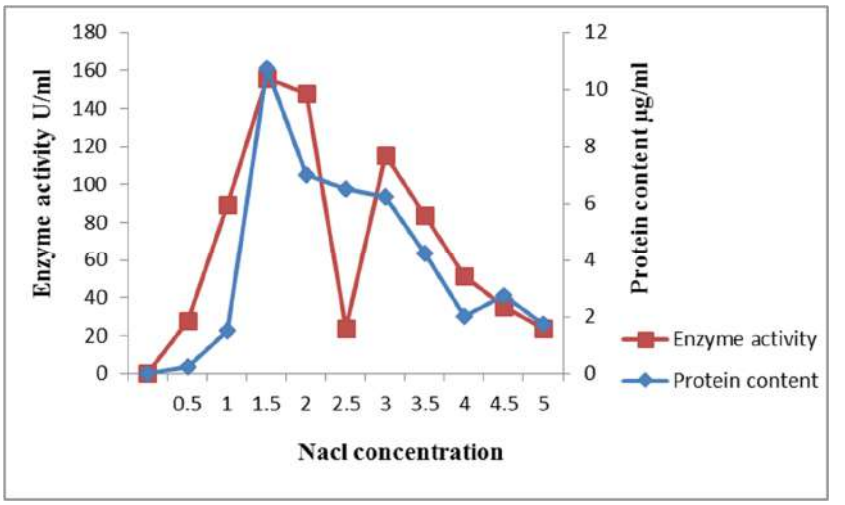

Figure 8: Effect of NaCl concentration on Enzyme activity and Protein content

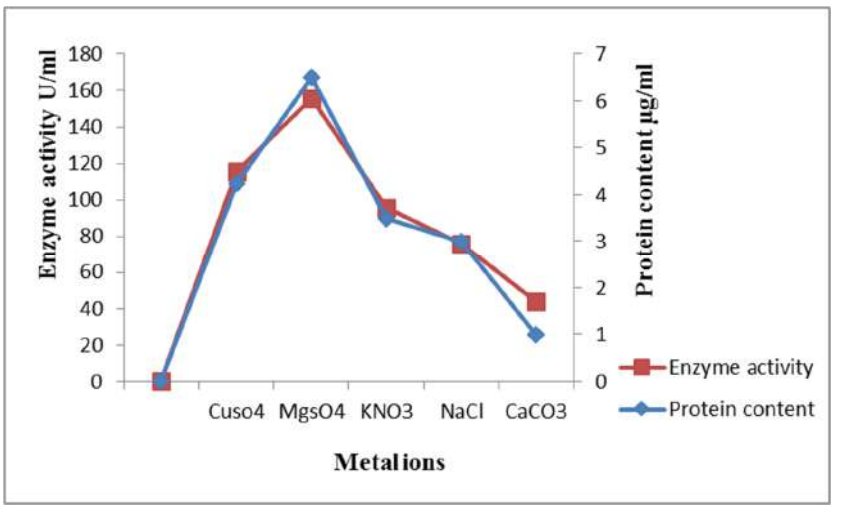

Figure 9: Effect of Metal ions on Enzyme activity and Protein Content

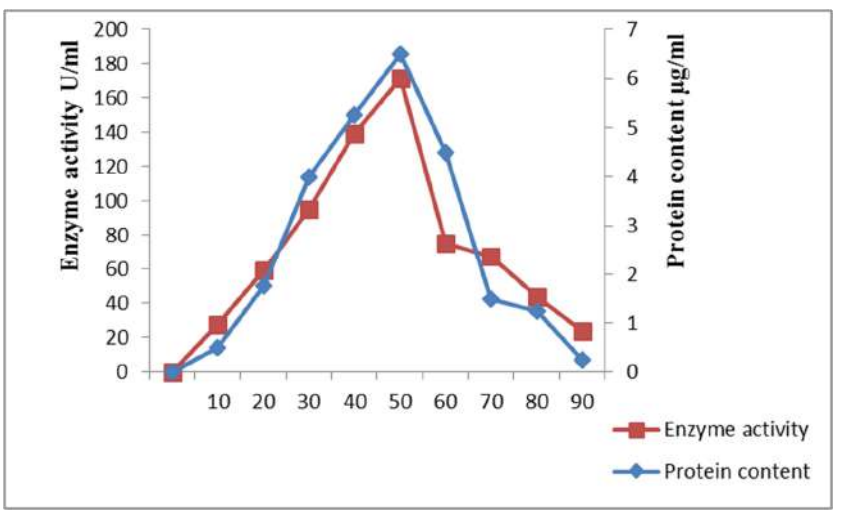

Figure 10: Effect of Substrate concentration on Enzyme activity and Protein Content

RESULTS AND DISCUSSION

The isolated strain was subjected to 16s RNA analysis and found to be Streptomyces gancidicus_ASD-KT852565. On optimization the enzyme activity of amylase was found to be gradually increasing with increase in temperature and the maximum was maintained at 40°C in Figure1. Thus reveals the organism to be mesophilic.

Secondly, maintaining the temperature the study on pH Figure2 revealed that increase in the enzyme and protein content was obtained in the neutral pH of 7.0 and gradually the production decreased with increase in pH.

Investigation with carbon source, one among the essential nutrients when added at the concentration of 1% the amylase growth was maximum with sucrose followed by lactose and least production with that of fructose was obtained in Figure3. The sucrose usage by the Streptomyces gancidicus_ASD-KT852565 indicates the simple sugar as its carbon source.

One of the other major nutrients, nitrogen source was added in 1% concentration and the optimized was obtained in Figure4 with Beef extract followed by nutrient broth, and the third position was shared by both urea and peptone.

Maintaining the optimized parameters, the influence of amino-acids was studied revealing the consumption of L-asparagine was highest followed by L-alanine and lowest consumption was found with L-tyrosine in Figure5.

The space for the culture is determined by the volume of the medium in the flask. In the present study the when the volume of the medium was at 50 ml per 250 ml flask as in Figure6 the enzyme production and activity found to be maximum.

The age of the culture determines the maturity of the organism in producing the enzyme. In this case Figure7 showed that 96 hour old fermented culture showed good growth after which increase in incubation led to the destruction in the activity and also protein content.

To study the salinity of the strain the NaCl concentration was varied and the yield increased when the NaCl concentration was maintained to 1.5 % as shown in the Figure 8.Gradual increase in the NaCl concentration resulted in the decrease the yield.

Metal ions acts as one of the trace elements which also help in enhancing the growth of the organism among the five metal ions used for investigation Figure9 depict $CuSo_4$ to be the highest amylase enhancer while $CaCo_3$ found to be the least enhancer of amylase by Streptomyces gancidicus_ASD-KT852565.

The amylase production by the organism is directly proportional to substrate utilization in the medium. In the present study starch was used as the substrate and the concentration of the substrate was found to be 50ml /Liter as shown in Fig 10 above which there was a gradual decrease in the enzyme activity with increase in the starch concentration.

CONCLUSION

Streptomyces is one the most widely present actinomycetes but, study of actinomycetes from marine soil on amylase is quite less. The present study revealed that the Streptomyces gancidicus_ASD-KT852565 is optimized by different parameters and is ready to be used in the industrial application of amylase production and also in various starch hydrolysis, detergents and paper industries etc. This results further leads to new fermentation parameters and easy recovery of the amylase in short time. All the ingredients utilized by the Streptomyces gancidicus_ASD-KT852565 also showed to be easily available and simple nutrients which can be readily degraded. Even though the organism was collected from marine soil sample the results prove it to be with anonym's character like mesophilic by its temperature where many marine organisms are thermophilic, neutral pH others are alkaline and the last with just 1.5 % NaCl concentration with normal salinity compared to other highly saline marine organisms. All this different characters make Streptomyces gancidicus_ASD-KT852565 to be unique.

ABBREVIATIONS

Abbreviation	Full Description
°C	degree
%	percentage
g/L	grams per litre
i.e.	that is
etc.	extra
µg/mL	Micro grams per millimetre
UV	Ultra violet
ml	millilitre
hrs.	hours

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