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Research Article

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Coconut oil cake -A novel substrate of solid state fermentation for the production of α -amylase using *Streptomyces spp.*

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ABSTRACT

In this present study Streptomyces was isolated from the Mangroves soil and identified by gram staining, biochemical tests and microscope observation. The optimum growth condition for the isolated organisms for the α-amylase production were such as pH, temperature, substrate concentration. On the production α- amylase using the isolated organisms the four different substrate such as under varying condition of coconut oil cake, groundnut oil cake, wheat bran, rice bran,were assessed coconut oil cake was produced the maximum amount of α-amylase in, pH4(18.5±0.02) *temperature25*^{0}C(8.5±0.02)*substrate concentration 15g* (12.5±0.02)*.Thus from the above study, it is concluded that coconut oil cake produces higher amount of α- amylase compared to groundnut oil cake, wheat bran, ricebran. Thin layer chromatographic the end products librated by to action of α-amylase to separate the glucose and maltose by using thin layer chromatographic.*

Keywords: α-Amylase**,** *Streptomyces*, Coconut oil cake, Groundnut oil cake,Wheat bran, Rice bran .

INTRODUCTION

Enzymes are ubiquitous components of all types of living cells, be it plants, animals or microbial growth. Enzynes are physiologically necessary for living organism. They are single chain or multiple chain protein and act as biocatalyst with the ability to promote specific chemical reaction under mild condition in most living organisms [1].

Recent development in biotechnology, particular an areas such as protein engineering and directed evolution have provided important tools for the efficient development of new enzymes. Enzymes found in nature have been used since ancient times in the production of food product, such as cheese, beer, wine and vinegar and in the manufacturing of commodes such as leather, indigo and liner [2].α- Amylase are enzyme, which hydrolyzes starch molecules given divers products including dextrin and progreesively smaller polymers composed of glucose units [3].The α- Amylase is a starch hydrolyzing enzymes which has many applications in various fields. It is used is breueries pharamaceuticals, chocolate industries etc.[4]

α-Amylase can be divided into two categories catalyze hydrolysis in a random manner in the interior of the starch molecule producing liner and branched oligosaccharides of various chain lengths. Exomamylases act from the non-reducing and successively resilting in short end production [5].A variety of microorganisms including bacteria, fungi, actinomycetes and have been reported to produces amylolytic enzymes. α – Amylases are used in a variety of industrial processes which require efficient of raw starch [6]. Actinomycetes however there have been few reports about the control of extracellular alpha amylase production by with respect to the suggested mechanism of α-amylase synthesis [7].

Solid – state – fermentation was carried out using coconut oil cake as substrate for the production of α –amylases.(8) Raw coconut oil cake supported the growth of the culture, resulting in the production of α –amylases in 24 hours. Process optimizing using a single parameter mode showed enhanced enzyme titer mode showed enhanced enzyme which was maximum when SSF carried out at for 72 hours using a substrate with 68 initial moisture. Coconut oil cake contains starch, soluble sugars. Soluble proteins, lipid and trace amount of nitrogen. Supplementation with glucose and starch further enhanced 0.5% starch. However, maltose inhibited the enzyme production studies on the enzyme production. Studies on the effect of addition external organic and inorganic nitrogenous compounds further should a positive impact on enzyme synthesis by the culture. Increase of 1-7 folds in the enzymes activity was obtained when peptone at 1% concentration was added to the fermentation medium. Use of coconut oil cake as raw material for enzyme synthesis could be of great commercial significance .Hence the present study was under taken by Coconut oil cake a novel substrate of solid state fermentation for the production of α- amylase using *Streptomyces spp.*

EXPERMENTAL SECTION

Isolation of Actinomycetes:

Isolation of Actinomycetes was performed by plating techniques using starch casein ager in medium (starch 10g, casein0.1g Ferrus sulphate 0.05g, Sodium chlornate 0.02g, Potassium nitrate 2g, Megnessium sulphate0.05g, sodium chloride 2g, Ager 20g, Distilled water 1000ml). The medium was prepared and sterilized at 121^0 c in 15bs pressure for 15 minutes. Then it was supplemented with griseofulvin and streptomycin to prevent the bacterial and fungal growth. The medium was poured into the sterile petriplate. The collected soil samples were diluted spread over the agar plates. The inoculated plated were incubated at 28 ± 2^0 C for 7-10 days. After incubation actinomycetes colonies were observed on the used for further investigated .[9]

Screening for α-Amylase actvity

The screening of actinomycetes isolated for α-amylase production was performed by spot inoculation method .The actinomycetes isolate was spot inoculated on sterile starch agar supplement with grisofulin 50µg/ml Soluble starch- 10.0, Polypeptone- 5.0 , KH_2 PO_4 -1.0, $MgSO_4$ $7H_2O$ - 5.0 , Agar- 15g . The plate was incubated at 50°C, for 48h after incubation the plate was flooded with iodine.Zone of hydrolysis and diameter of colony was recorded[10]

Production in Solid State Fermentation

The static Experiments were conducted in 250ml of substrate moistened with mineral salt solution of the following composTrisodium citrate-2.5, KH_2 PO_4 -5.0,NH_4NO_3 -2.0 (NH_4) SO_4-4.0 $MgSO_4$0.2 to 50% moisture Content Flakes were plugged with cotton and sterilized by autoclaving for 15 minutes at 121^0C. After sterilization, the flasks were cooled and inoculated with 10% inoculums. The flasks were incubated at 30 ± 1^0C and initial pH5 under still culture conditions for a desired period. The contents of the flasks were gently shaken after every 12 hours.[11]

Enzyme Extraction

To the fermented laugh 50mm citrate buffer 1:10 ratio was added and homogenized for 24 hours with a constant stirring at room temperature. This suspension was filtered through what mann filter paper number1 and the filtrate was again centrifuged at 6000 rpm for 15minutes. This solid free supertant was used as enzyme source for assaying α-amylase activity.[12]

Optimization of the production process

The solid state fermentation of the selected substrate coconut oil cake, groundout oil cake and rice bran, wheat barn were optimized by varying process. Condition like pH, temperature, carbon nitrogen additives and substrate concentration. The traditional classical method involved varying on parameter at a time by maintaining pre-optimized solid state fermentation .[13]

Estimation of Protein by Lowry's Method [14]

500mg of copper sulphate was dissolved in 100ml of 1% sodium potassium tartarate.2g of Sodium bicarbonate was dissolved with distilled water and made up to 100ml with 0.1N NaOHIt was freshly prepared each time just prior to use, by mixing 50ml of alkaline sodium carbonate with 1ml of copper sulphate sodium potassium tartarate solution. Commercially available Folin's reagent was diluted at 1:1 ratio with distilled water just prior to use.

Procedure

0.5ml of culture filtrates sample was taken in a test tube and to add 4.5 ml of Lowry's reagent. Then 0.5ml of Folin's reagent was added. It was inclubated at 30 minutes. The absorbance was read at 680nm.

Total amount of protein = $\frac{\text{OD of the sample value}}{\text{OD of the Standard value}}$ X 100

Statistical analysis[15]
Mean

Mean (x) = $\sum x/N$

Standard Deviation

Standard Deviation $\sigma = \sqrt{\frac{\sum(X - X)^2}{N - 1}}$

RESULTS AND DISCUSSION

The α- Amylase producing *Streptomyces was* isolated for the production and performed by spot inoculation method. The *Streptomyces* isolated agar supplemented with grisofulvin 50µg/ml. The plate was incubated at 50^0C for 48h. After incubation the plate was flooded with iodine. Zone of hydrolysis of starch around the four different tested, the maximum enzyme activity was observed at pH 4, in α- Amylase activity (18.5±0.02) and protein content was (6.7±0.01) α- Amylase at among the four different temperature tested the maximum α- Amylase yield coconut oil cake enzyme activity was observed at temperature in α- Amylase (8.5±0.02) and protein content (8.6±0.002 1v/ml). Among the four different substrate concentration tested, the maximum enzyme activity was observed at substrate concentration 15g in α-amylase (12.5±0.02) and protein content was (205±0.02). The total amount of protein present in the purified enzyme was estimated by Lowry's method. The total amount of protein in the purified enzyme was (13.06±0.01).A potential method to convert coconut oil cake into a value added product such as animal feed supplement and production of enzyme.[16] The effect of supplementation of coconut oil cake with an additional carbon source soluble starch did not show any yield impact on α-amylase production. In fact the yield were reduced even with its lowest concentration at 0.5% starch and highest starch concentration.

Table-1

Tests	***Stretomyces***
Gram Staining	Gram Positive
shape	Rod
Indole Test	+
Methyl Red Test	+
Voges – Prosouer Test	+
Citrate utilization Test	_
Catalase Test	_
H_2S production Test	+

Table-2

Organisms	**Substrate**	**Enzyme Activity U/ml p^H**			
		3	**4**	**5**	**6**
Streptomyces	Coconut oil cake	18.5±0.02	3.5±0.02	6.5±0.01	4.5±0.031
	Ground nut Oil Cake	1.2±0.07	1.5±0.08	5.2±0.04	5.5±0.04
	Wheat bran	6.5±0.03	5.0±0.06	2.5±0.07	3±0.02
	Rice Bran	3.0±0.00	2.5±0.05	17.5±0.01	2.5±0.01

Values are expressed as Mean ±Standard deviation

Table-3

Organisims	**Substrate**	**Enzyme Activity U/ml Temperature**			
		25^0C	**35^0C**	**45^0C**	**55^0C**
Streptomyces	Cocunt Oil Cake	8.5±0.02	7.5±0.03	3.8±0.03	9.5±0.04
	Ground nut oil cake	1.2±0.04	1.5±0.01	1.2±0.04	1.2±0.02
	Wheat Bran	6.2±0.06	1.5±0.03	2.5±0.01	4.5±0.03
	Rice Bran	5.5±0.01	1.3±0.03	6.5±0.05	3.2±0.01

Values are expressed as Mean ±Standard deviation

CONCLUSION

Thin layer chromatographic the end products librated by the action of α-amylase on starch were identified by spotting starch digest and to separate the glucose and maltose by using thin layer chromatographic. On the basis of the results of the present study, it is concluded that the utilization of coconut oil cake, groundnut oil cake, wheat bran, Rice bran as a solid substrate could lead to large scale production of industrial enzyme and also contribute to safe and economic waste management in the environment, where these wastes are continuously accumulated, and cause pollution problems.

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