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RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF AMYLASE ENZYME FROM LICHEN *PARMELIA* SP COLLECTED FROM ARUNACHAL PRADESH

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ABSTRACT

The plant material (lichen) was collected from the hills of the Arunachal Pradesh and it was identified as the species of the genus *Parmelia*. The lichen was crushed in the extraction buffer in order to isolate the enzyme. The extracted material was first tested for the presence of the protein by Lowry's method which showed positive result. The extracted material was now tested for the presence of the enzyme amylase. 1% starch solution was used as substrate in order to test the activity of amylase. In our first experiment we wished to optimize the amount of the crude extracted enzyme, amylase from the plant material. Here we used different volumes of the extract i.e 1.0 ml, 2.5 ml, 5.0 ml, 7.0 ml and 10.0 ml [Table 1]. From the observation it could be inferred that the optimum volume of crude extract was 5ml. This is because as we increased the volume above 5ml the activity of the enzymes didn't showed any significant increase. Therefore we used 5ml of crude extract in our further experiments. We tried to find out the time of incubation for the maximum activity of the enzyme.

The time of incubation was varied from 1min to 20 min. [Table 2]. The activity increased from 1min to 10 min. But we found that from 15mins onwards the amount of starch hydrolyzed started to decrease. However we were not able to cover the short time intervals between 10 min to 15mins which may show the maximum activity. Therefore, we optimized the incubation period of 10 min only for our further experiments. The use of any enzyme for the commercial purpose depends on the temperature in which it shows its maximum activity. Our next aim was to optimize the temperature for the maximum activity of the crude extracted amylase from the foliose lichen. In our experiment a temperature range of 5^0C to 55 ^{0}C was maintained [Table 3].

It was observed that at pH 6 and at incubation time of 10 min. the enzyme showed its maximum activity at a very low temperature of 15^0C. A drastic fall of enzyme activity was recorded as we increase the temperature from 25^0C onwards. However, a slight increase in activity was seen at 55^0C. This observation can be compared with the occurrence of the foliose lichen that was collected from the hills of Arunachal Pradesh at a low temperature of 10-15 ^{0}C.

This low temperature activity of the amylase therefore can be used for the industrial purpose. For an enzyme activity, the pH is an important factor. So our next aim was to optimize the pH for the enzyme activity. The different pH solutions were used from pH 3.0 to pH 10 [Table 4]. It was found that the enzyme showed maximum activity at pH 7.0 while the activity is less at acidic and basic pH compared the neutral pH.

Further we tried to purify the enzyme amylase by giving the ammonium sulphate cut at different concentrations, which ranged from 1.5 M to 4 M [Table 5]. It was found that the amylase showed maximum activity at an ammonium sulphate concentration of 1.5 M and 2 M. To remove the ammonium sulphate dialysis [Table 6] was done for further purification. It was observed that 0.1ml of the enzyme showed the activity comparable to the crude enzyme extract keeping all the parameters constant such as pH 7.0, incubation period of 10mins and temperature of 15°C. For the purpose of purification salting out was done by ammonium sulphate.

The trace of ammonium sulphate was removed from the protein by dialysis. The activity of the enzyme both in precipitated state in ammonium sulphate and after dialysis were measured and found that the activity has increased significantly [Table 7], with respect to the crude enzyme. Lastly the partially purified enzyme amylase from the foliose lichen, *Parmelia* was studied to observe the effect of metals on enzyme activity. This was done to see the role of metals as metals behave as the cofactors for the enzyme activity. The different metals used were calcium, potassium, sodium, copper and iron [Table 8]. With Cu, we found that at very low concentration it increased the activity however it inhibited the activity at higher concentration. So, Cu is not desirable in the production process. Ca and Fe, however showed increase in the enzyme activity and are therefore desirable metals for the large scale production of the enzyme.

Therefore all these results interpret that the enzyme amylase isolated from the foliose lichen showed its optimum activity at the low temperature of 15°C, at an incubation period of 10mins with Ca and Fe as its metal inducers. This low temperature nature of the enzyme amylase can be exploited in future for its commercial application.

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INTRODUCTION:

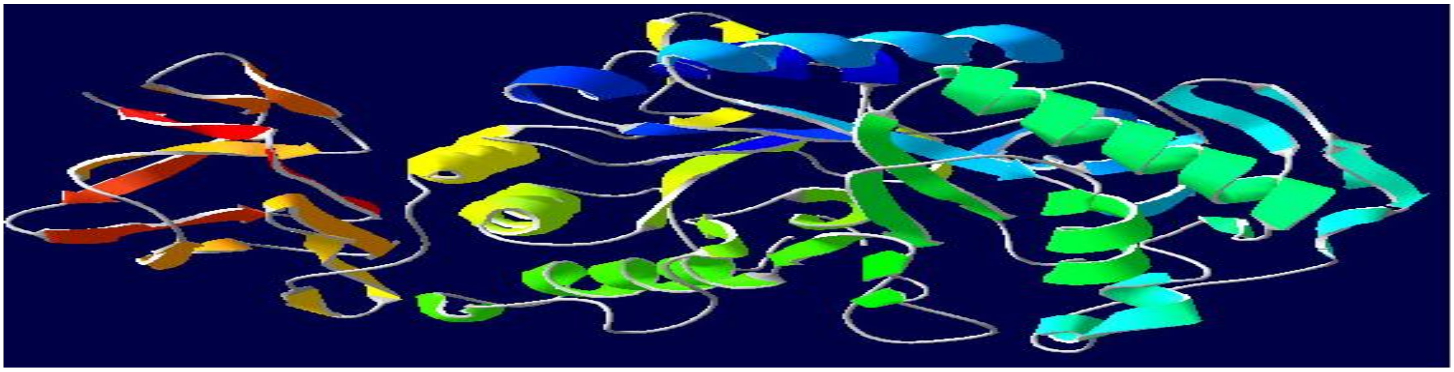
α-Amylases (E.C. 3.2.1.1) are glycoside hydrolases that have been classified within family GH13 [1]. They play an important role in starch degradation and represent about 25 to 33% of the enzyme world market, in second place after proteases. They specifically catalyze the endohydrolysis of 1, 4-α-D-glucosidic linkages of starch and related polysaccharides to produce oligosaccharides of different sizes. α-Amylases constitute an important class of enzymes that find many biotechnological applications in processes requiring the degradation of starch [2], such as baking, brewing, deterging [3], and fabric desizing (in textile industries). Most α-amylases produce glucose or maltose as the major end products of starch hydrolysis. However, amylases that specifically produce malt oligosaccharides from starch have been reported [4]. These include maltohexaose producing amylases from *Bacillus stearothermophilus* US100 and *Bacillus amyloliquefaciens,* and maltopentaose producing amylase from *Bacillus cereus* NY-14 [5]; maltotetrose-producing amylases from *Pseudomonas stutzeri* and *Bacillus circulans*, and maltotriose-producing amylases from *Streptomyces griseus* NA-468, *Bacillus subtilis* [6], and *Microbacterium imperiale* [7]. A number of amylases have been reported with different molecular weights, optimum pH, thermo stability, and metal ion dependence [8, 9, 10, 11]. Amylases are universally produced by prokaryotes and eukaryotes, including plants and animals. However, only a few species of fungi and bacteria are used for the commercial production of α- amylases because of their ease of cultivation and the desirable physicochemical properties of the secreted enzymes [12, 13]. Filamentous fungi possess a number of features that make them attractive for scientific as well as industrial purposes.

Among the diverse microorganisms producing amylase, α amylases from *Bacillus* sp. have been the most extensively studied, partly due to their extreme thermo stability, while other bacterial amylases with special properties are also continually reported [14] However hemophilic and hemophilic fungi are also important source of amylolytic enzymes due to their different properties as to bacterial enzymes, including optimal working conditions and reaction specificity. These enzymes are widely used in many applications, including the food, feed and fermentation industries. Detailed studies on amylolytic enzymes have been largely limited to a few species of mesophilic and thermophilic fungi. [15, 11] especially *Aspergillus* sp., which have been studied in terms of theory ad application [16]. In addition a number of new fungal isolates producing amylolytic enzymes have also been reported recently, e.g., *Rhizomucor pusillus* [17], & *Trichoderma* sp [18]. Accentuating academic and industrial interest on amylases. Driven by the search for new amylolytic enzymes with desirable industrial properties, including improved thermostabilty, novel reaction specificity, and low inhibition by glucose, the product of the saccharification process, the exploration of the new fungi as a source of potent starch converting enzymes is thus of interest.

Endophytic fungi are considered potent microbes producing hydrolytic enzymes targeting plant derived macromolecules. Considerable interest has been devoted to ascomycetes in the family *Botryosphariaceae* as to enzyme production and characterization [19, 20]. Amylolytic enzyme production from *Botryodiplodia theobromae* grown on different agricultural residues has also been reported [21, 22]. The BIOTEC Culture Collection (BCC) holds one of the world's largest fungal collections, which is a promising source for industrial enzyme discovery. From an extensive screening of fungal isolates collected in BCC, an endophyte *Fusicoccum* sp.BCC4124, an asexual state of *Botryosphaeria* sp., was identified for its strong thermotolerent amylolytic activity.

In this study, the production, inducibility, biochemical characterization of α- amylase from the lichen *Parmelia sp*. has been done. This study may contribute to understanding of the diversity of α-amylase from different sources and their application for industrial purpose; accentuate the potential of biodiversity wealth as the source of new enzymes of industrial and academic interest

Figure 1: Amylase – 3d Structure



MATERIALS AND METHODS:

The material used as the enzyme source is a foliose lichen collected from the hills of **Arunachal Pradesh; Bomdi-La** . The lichen belongs to the genus ***Parmelia***

CHEMICALS:

1. Phosphate buffer saline (PBS)
2. 10% Glycerol
3. Charcoal powder
4. Sodium Acetate buffer
5. 1% soluble starch
6. Nelson's Reagent
7. Arsenomolybdate color reagent
8. Protein reagents (Lowry's method)
9. Folin phenol
10. Tris HCl buffer
11. Ammonium sulphate
12. Sodium Chloride
13. Potassium hydroxide
14. Calcium Hydroxide
15. Ferrous sulphate
16. Copper sulphate

All the above chemicals were of the make Merck Limited, Qualigens Fine Chemical Company, a division of Glaxo Smith Kline Pharma. Ltd.

INSTRUMENTS USED:

1. Cold Centrifuge "Remi-C-24"
2. Digital balance (Adair Dutt Instruments PVT.LTD.)
3. Spectrophotometer (Systronics-1) and Visican-167 [2]. Spectrophotometer (104)
4. Refrigerator
5. Digital Thermometer
6. Digital pH pen
7. Deionizer water maker Anion Exchanger & Cation Exchanger- Demineralizer -Zeolite India PVT.LTD. Glass distilled water maker (Qualigens Fine chemicals-Operation-230,V.A.C.-50/60 H_2 Single phase, Model no.-BSIC ECO.4, SL.NO.-0301506-Bham Scientific Instruments CO.RIT.LTD.

PREPARATION OF PHOSPHATE BUFFER SOLUTION:

REQUIREMENTS:

NaCl	8gms
KCl	0.2 gms
Na_2HPO_4	1.44 gms
NaK_2PO_4	0.24 gms

PROCEDURE:

8gms Sodium chloride,0.2 gms Potassium Chloride, 1.44 gms Di Sodium Hydrogen Phosphate,0.24 gms Potassium Di Hydrogen phosphate were dissolved in 800ml. distilled water & then pH adjusted to 7.4 by adding HCl. The final volume is made up to 1litre by adding distilled water [23, 24].

PREPARATION OF EXTRACTION BUFFER:

Phosphate buffer saline was added to 10% glycerol in 9:1 ratio to prepare the extraction buffer for enzyme isolation.

PREPARATION OF SODIUM ACETATE BUFFER (pH-6):

Dissolving 8.2gm of CH_3COONa in 100ml distilled water, 1M CH_3COONa was prepared. 6ml acetic acid (100%) was dissolved in distilled water to make up the volume to 100ml which makes 1M acetic acid. 17.37ml CH_3COONa & 1ml acetic acid were mixed and volume was made up to 100ml. to prepare 1M Sodium acetate buffer. Adjust the pH to 6.0 by adding 1N NaOH solution.

Figure 2: The foliose lichen on bare rock surface

PREPARATION OF NELSON'S REAGENT:

REAGENT A:

Sodium Carbonate	25 gms
Rochelle Salt (Na-K Tartrate)	25 gms
Sodium bi Carbonate	20 gms
Sodium Sulphate	200 gms

All the reagents were dissolved in 800ml distilled water; volume was made up to 1000ml. If precipitate appears it was filtered before use.

REAGENT B:

15 gms of Copper Sulphate was dissolved in 100ml distilled water (15% $CuSO_4$).To it 2-3 drops concentrated H_2SO_4 was added.

PREPARATION OF ARSENOMOLYBDATE COLOUR REAGENT:

To make 500 ml of the reagent, 25 gms of Ammonium Molybdate was dissolved in 450ml. distilled water, 21ml. of concentrated H_2SO_4 was added, 3gms. $Na_2HSO_4{:}7H_2O$ was dissolved by adding additional 25ml. distilled water. Incubate this reagent at $37^{\circ}C$ for 24-38 hours [25].

PREPARATION OF LOWRY'S REAGENTS:

Lowry A

Sodium hydroxide pellet	0.4 gms
Sodium Carbonate	2.0 gms
Distilled water	100 ml

Lowry B

Copper Sulphate	500 mg
Sodium –Potassium tartarate	1 gm
Distilled water	100 ml

Lowry A & Lowry B in 50:1 ratio was mixed thoroughly to prepare Lowry's reagent [26]

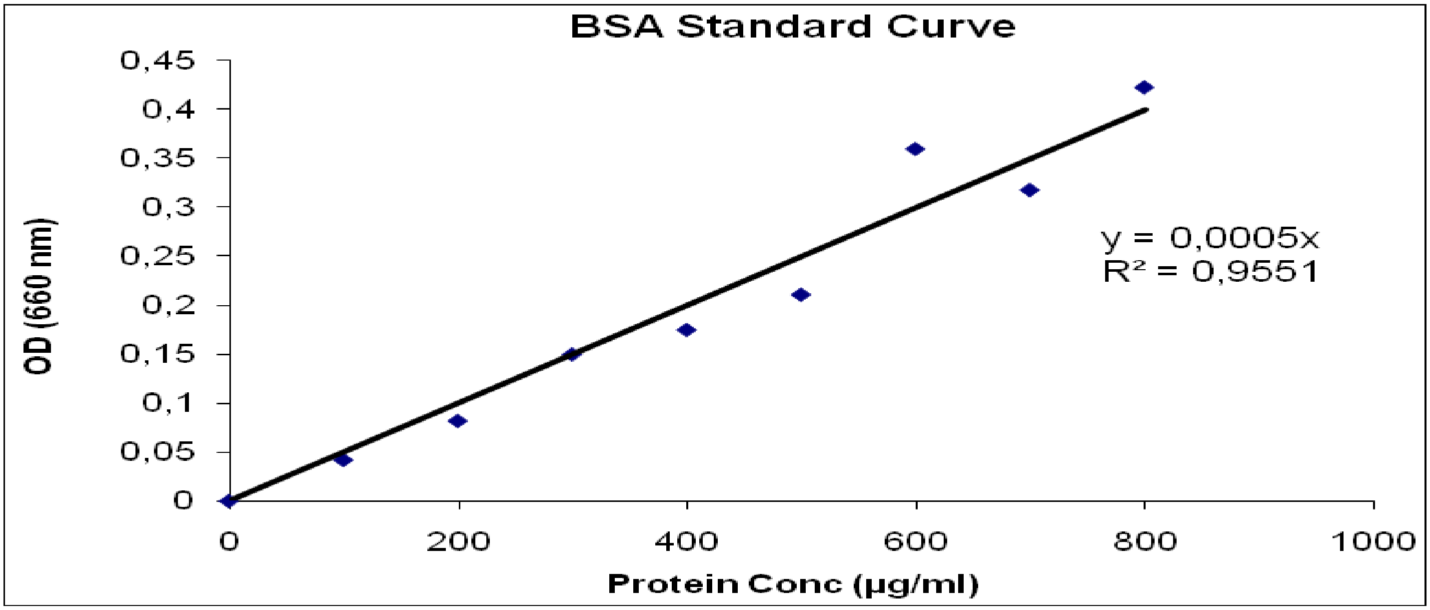
PREPARATION OF ENZYME EXTRACT:

To prepare enzyme extract foliose lichen *Parmelia sp.* collected from Bomdi-La, West Kameng District, Arunachal Pradesh was used as the source of isolation. The freezed plant material was thoroughly washed in tap water, dried it by blotting paper and finally weighed to desired amount by Pan Balance in a dry container. By using minimum volume of PBS buffer pH 7.4 it was crushed well in mortar & pestle. Then it was centrifuged at 10,000 r.p.m. for 10min. Taking the supernatant (discarding pellet) in a test tube various experiments were performed.

ESTIMATION OF PROTEIN OF THE LICHEN SAMPLE USING LOWRY MIXTURE:

Weighed 1gm of foliose lichen by pan balance in a dry container, then it was thoroughly washed in tap water, dried it by blotting paper. Then by using minimum volume of PBS. Buffer it was crushed well in mortar & pestle. Then it was centrifuged at 10,000 r.p.m. for 10mins. The supernatant (discarding pellet) was collected in a test tube. The final volume was made up to 10ml. using PBS buffer. Then by taking 0.2 ml enzyme extract, 1.8 ml PBS buffer and 2ml Lowry's reagent, it was incubated for 15 minutes. 0.5ml. folin phenol (diluted by distilled water in 1:45 ratio) was added & again incubate for 30min. O.D. was recorded at 660nm using spectrophotometer.

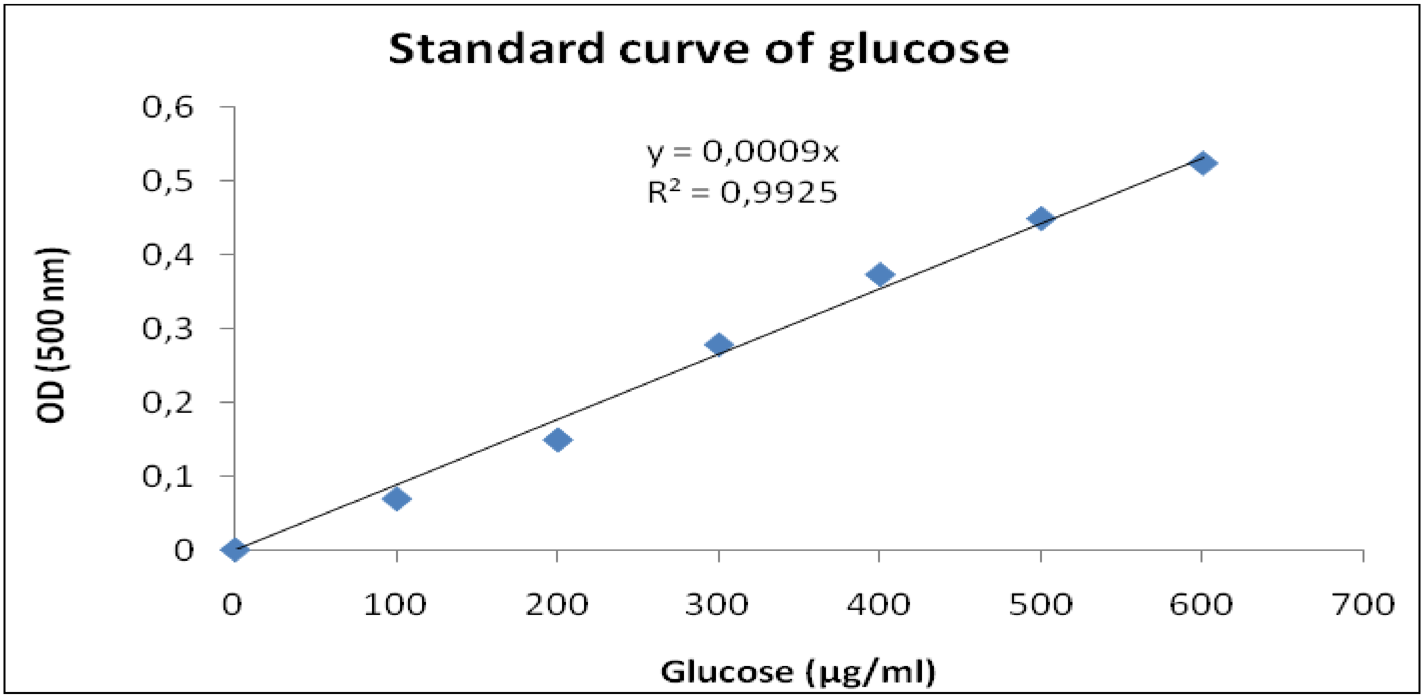
Figure 3: BSA Standard Curve



PREPARATION OF STANDARD CURVE USING STANDARD GLUCOSE SOLUTION [1000MG/ML]:

100mg. of glucose weighed and dissolved it in 100ml distilled water. Then made the concentration gradient of 100µg/ml to 1000µg/ml by adding 1ml,-10ml standard glucose solution in different test tubes respectively and add respective volume of distilled water. Make triplet of the same. Then take 1ml from each and add to them 2ml Na- acetate buffer and 1ml Nelson reagent. Then heat it for 20 mins. & cool the reagent mixture. Then add 1ml Arseno molybdate reagent to them to develop colour. Dilute it to 5ml by adding distilled water and measure color at 500n.m.

Figure 4: Standard Curve of Glucose



VARIOUS PARAMETERS AFFECTING AMYLASE ACTIVITY:

1. ENZYME EXTRACTS CONCENTRATION:

Taking 4gms of lichen sample extraction was done according to above stated procedure. The volume of enzyme extract made up to 40ml. 5 reaction sets were prepared taking 1ml, 2.5ml, 5ml, 7.5ml, 10ml of enzyme extracts & to them 14ml., 12.5ml., 10ml, 7.5ml, 5ml. sodium acetate buffer was added respectively to make the final volume to 15ml. 5ml 1% starch solution in each 5 test tubes was added. In control set instead of crude extracted enzyme, only extraction buffer was added. All the sets were incubated for 15 minutes at temperature of 25°C. Then 1ml of aliquot was pipetted out & to it 1ml of Nelson's reagent was added to stop the reaction. the mixture was exposed to the hot water bath for 15-20mins. & then cooled, 1ml arsenomolybdate color reagent was added, dilute it adding distilled water up to 25 ml & OD by spectrophotometer at wavelength of 500 n.m.

2. TIME:

Taking 4gms of lichen sample extraction was done according to previous procedure. Then volume of enzyme extract was made up to 40ml. 5 reaction sets were prepared taking 5ml of enzyme extract,10ml Sodium acetate-acetic acid buffer,5ml 1% starch solution in 5 test tubes. In control set instead of crude enzyme, only extraction buffer was added. The sets were incubated for 1min., 5min., 10min., 15min., and 20min. Then 1ml of aliquot was pipette out & to it 1ml of Nelson's reagent was added to stop the reaction. Heat the mixture in hot water bath for 15-20min. & then cooled it, 1ml arsenomolybdate color reagent was added. Dilution was done by adding distilled water up to 25 ml & OD was measured in spectrophotometer at wavelength of 500nm

3. TEMPERATURE:

Taking 4gms of lichen sample, extraction was done according to above stated procedure. Then volume of enzyme extract was made up to 40ml. Five reaction sets were prepared taking 5ml of enzyme extract, 10ml Sodium acetate buffer, 5ml 1% starch solution in 5 test tubes. In control set instead of crude extracted enzyme, only extraction buffer was added. All the sets were incubated for 10 minutes at various controlled temperatures of 5°C, 15°C, 25°C, 35°C, 45°C, and 55°C. Then 1ml of aliquot was pipetted out & to it 1ml of Nelson's reagent was added to stop the reaction. The mixture was exposed in hot water bath for 15-20 mins. & then it was cooled, 1ml arsenomolybdate color reagent was added. The test tubes were further diluted by adding distilled water up to 25 ml. A blue colored appeared & OD was measured by spectrophotometer at wavelength of 500nm.

4. pH:

Taking 4gms of lichen sample, extraction was done according to previous procedure. Then volume of enzyme extract was made up to 40ml. 5 reaction sets were prepared taking 5ml of enzyme extract & to each set gradually buffers of pH 3.0, 4.0, 5.0, 6.0 ,7.0 ,8.0,9.0 were added. Then to every set 5ml starch solution (1%)was added. In control set instead of crude extracted enzyme, only extraction buffer was added. All the sets were incubated for 10 minutes at a temperature of 25°C. Then 1ml of aliquot was pipetted out & to it 1ml of Nelson's reagent was added to stop the reaction. The mixture was heated in hot water bath for 15-20 mins. & then cooled it. 1ml arsenomolybdate color reagent was added. Further dilution was done by adding distilled water up to 25 ml & color development was measured in spectrophotometer at wavelength of 500nm

pH	Na-Acetate (ml)	Acetic acid (ml)	Volume make up to (ml)
3.0	1	57.54	100
4.0	1	5.75	100
5.0	1.74	1	100
6.0	17.37	1	100

Table 1: Preparation of Buffers of Various pH (A) 1M Na Acetate buffer:

pH	0.2M Tris (24.2gms in 1000ml distilled water) Soln. A in ml.	0.2N HCl (ml) Soln.B	Volume make upto (ml)
7.2	50	5	200
8.0	50	26.8	200
9.0	50	5	200

Table 2: Preparation of Buffers of Various pH (A) Tris HCl buffer:

PROCESSES USED FOR PARTIAL PURIFICATION OF AMYLASE ENZYME:

1. AMMONIUM SULPHATE CUT:

Ammonium sulphate is practically the only precipitant that is frequently used for fractionation of the crude extract (in a so called **ammonium sulphate cut** procedure). During precipitation the protein solution was kept on ice or at 4ºC to prevent proteins from denaturing due to heat produced by dissolving the ammonium sulphate. In our experiment 5 ml of the cold crude extract was taken and 3 ml of 4M ammonium sulphate solution was added to it .That gave 1.5M of ammonium sulphate**.** The solution was spin down for 2 min in the Remi cold centrifuge. The supernatant fraction was placed into a fresh tube and pellet was re-suspend in 5 ml of extraction buffer.

This tube was marked: **1.5M pellet.**

To the supernatant fraction 2 ml of 4M **ammonium sulphate** was added and previous step was repeated. That gave a pellet which was again re-suspended and marked as: **2.0M pellet.** To the supernatant fraction 660 mg of ammonium sulphate was added and the process was repeated as above. The re-suspended pellet was marked: **2.5M pellet.** The above process was repeated two more times to get 3M and 3.5M pellets and 3.5M supernatant.

Protein concentration was checked in each tube. It was found that 3.5M supernatant fraction had a very low protein concentration.

The activity for amylase was tested with each re-suspended pellet solution. [27]

2. DIALYSIS:

After the above step the protein sample would contain significant amounts of ammonium sulphate and could not be used directly for further experiments. So, an appropriate volume of the ammonium sulphate precipitate suspension was placed into a centrifugation tube and the precipitate was collected by centrifugation at 10,000 rpm for 10 min at 4°C. The supernatant fraction was removed carefully so those every last drops of ammonium sulphate solution were taken out. The pellet was re-suspending in a small volume of extraction buffer and dialyzed it against some 100-200 volumes of buffer. 2-3 changes were made and the last one was left overnight for full equilibration. [27].

TEST FOR METAL REQUIREMENT OF AMYLASE ENZYME :

Thoroughly dialyzed enzyme was used to study the metal ion requirement for the activity of the enzyme. The different metals used were calcium, potassium, sodium, copper and iron. The concentration used was 1mM, 5mM and 10mM. Each concentration was added separately to the reaction mixture and the reducing sugar formed in the standard assay condition was measured [28].

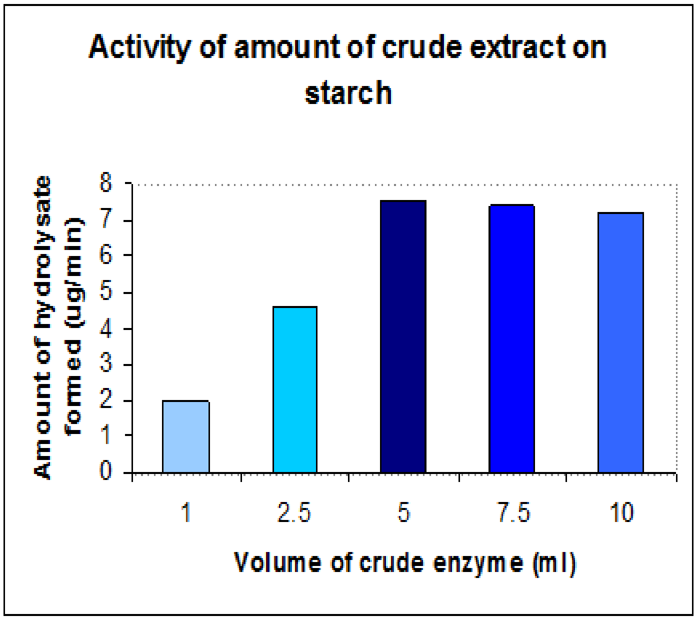
OBSERVATIONS:

1. ACTIVITY OF AMOUNT OF CRUDE EXTRACTS ON STARCH:

Crude enzyme extract	Amount of hydrolysate formed (µg/min)
1 ml	2
2.5 ml	4.6
5 ml	**7.5**
7.5 ml	7.4
10 ml	7.2

Table No: 3

Figure 5:



2. AMOUNT OF HYDROLYSATE FORMED BY 5ML CRUDE EXTRACT AT DIFFERENT TIME INTERVAL:

Volume of the crude extract in ml.	Duration (min)	Amount of hydrolysate formed(µg)
5	1	71
5	5	305
5	**10**	**386**
5	15	318
5	20	297

Table No. 4

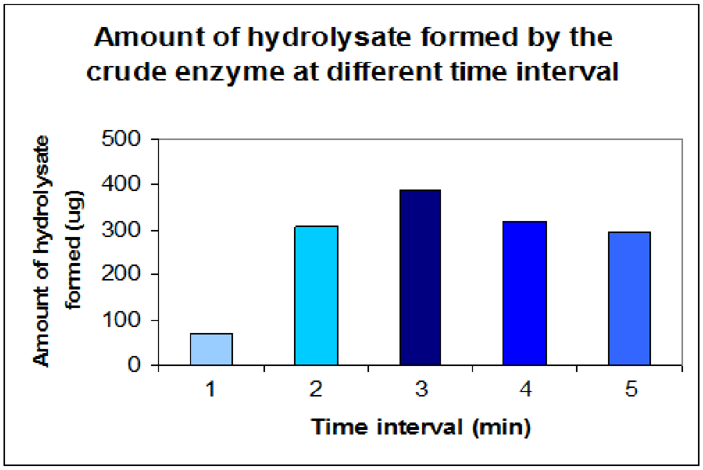


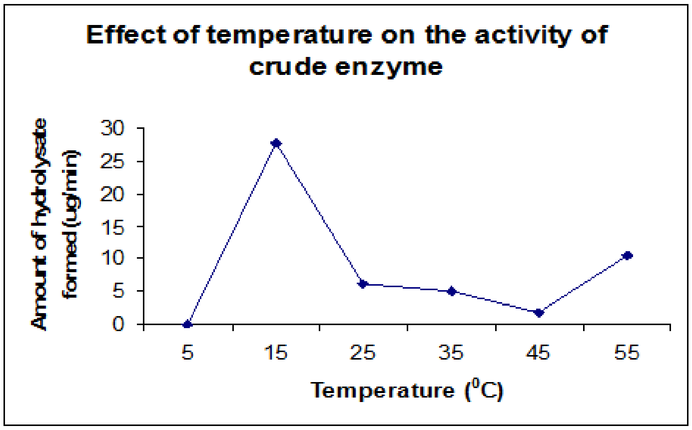
Figure 6:

3. Effect of temperature on enzyme activity

Volume of the crude extract in ml.	Temperature	Amount of hydrolysate formed (µg/min)
5	5ºC	0.1
5	**15ºC**	**27.7**
5	25ºC	6.2
5	35ºC	5.0
5	45ºC	1.9
5	55ºC	10.5

Table No. 3

Figure 7:

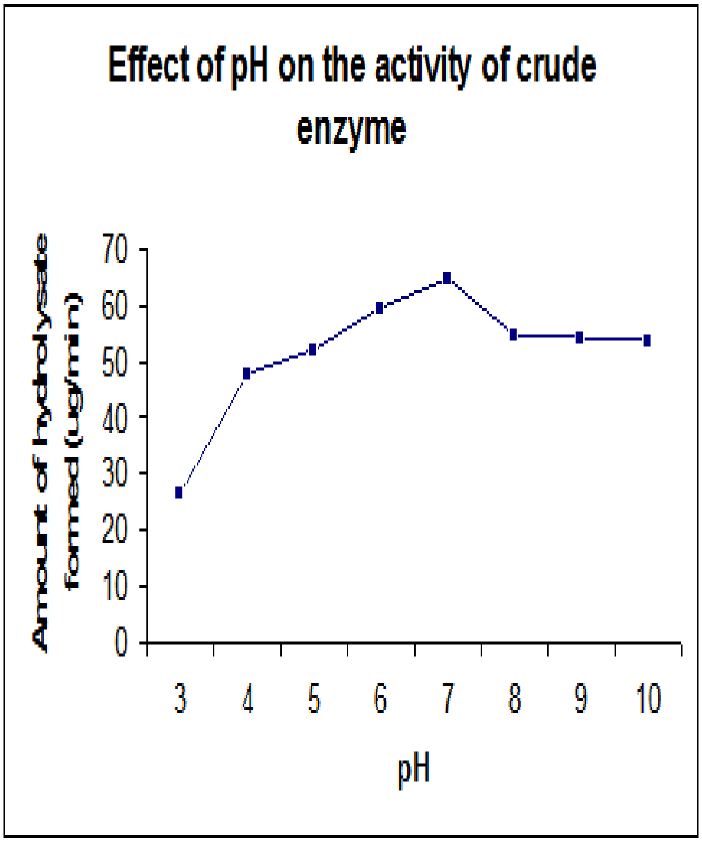


4. Effect of pH on enzyme activity:

Volume of the crude extract in ml.	pH	Amount of hydrolysate formed (µg/min)
5	3	26.2
5	4	47.9
5	5	52.3
5	6	59.7
5	**7**	**64.6**
5	8	54.6
5	9	54.1
5	10	53.9

Table 4:

Figure 8:

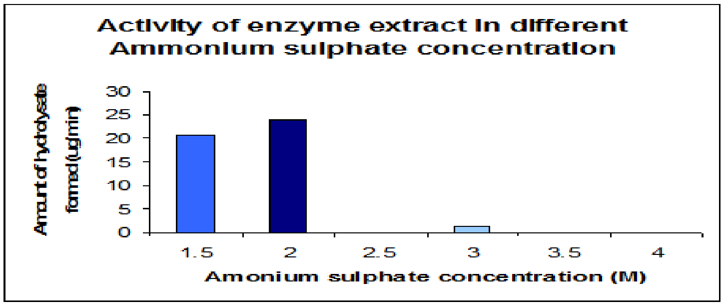


1. Ammonium sulphate concentration at which activity is observed:

Volume of the crude extract in ml	Ammonium sulphate conc. (M)	Amount of hydrolysate formed (µg/min)
1	**1.5**	**20.8**
1	**2**	**24.0**
1	2.5	-
1	3	1.2
1	3.5	-
1	4	-

Table 5:

Figure 9:



6. Effect of dialysed extract:

Volume of the crude extract in ml	Conc. from where dialysis is done	OD value	Amount of hydrolysate formed
0.1	1.5 M	0.915	91.5
0.1	2M	1.015	101.5

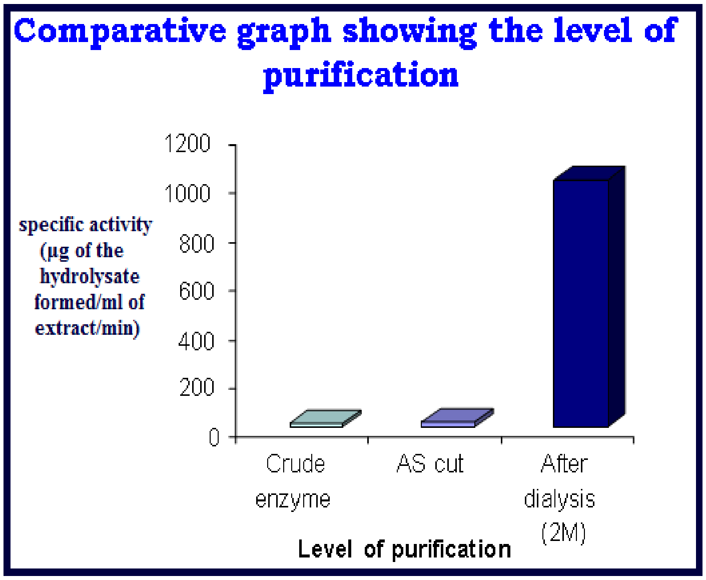
Table 5:

COMPARATIVE CHART SHOWING THE SPECIFIC ACTIVITY OF ENZYME AMYLASE AT DIFFERENT STATE OF PURIFICATION:

Level of purification	Volume of extract used (ml)	Activity(ug/ml/min)
1. Crude	**1**	**12.92**
2. Ammonium sulphate concentration at 2M	**1**	**24**
3. Dialysed	**1**	**1015**

Table 6:

Figure 10:

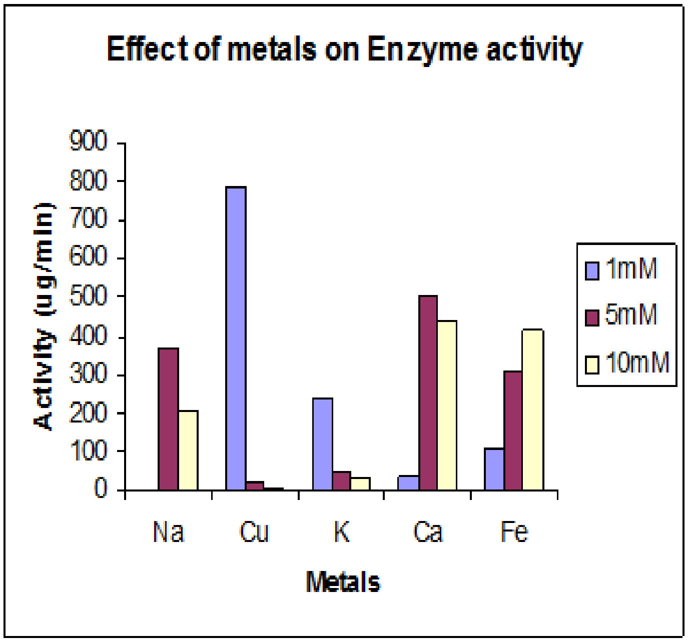


6. Effect of metals on Amylase enzyme activity:

Volume of the crude extract in ml	Metals	Con. used	OD value	Amount of hydrolysate formed
0.1	**Na**	1 mM	Low	-
0.1		5 mM	0.368	368
0.1		10 mM	0.207	207
0.1	**Cu**	1 mM	0.782	782
0.1		5 mM	0.017	17
0.1		10 mM	0.004	4
0.1	**K**	1 mM	0.242	242
0.1		5 mM	0.045	45
0.1		10mM	0.031	31
0.1	**Ca**	1 mM	0.034	34
0.1		5 mM	0.503	503
0.1		10 mM	0.438	438
0.1	**Fe**	1 mM	0.103	103
0.1		5 mM	0.307	307
0.1		10 mM	0.414	414

Table 8:

Figure 11:



FOLIOSE LICHEN: *PARMELIA* SP:

Figure 12:

RESULTS AND DISCUSSION:

The plant material (lichen) was collected from the hills of the Arunachal Pradesh and it was identified as the species of the genus *Parmelia*. The lichen was crushed in the extraction buffer in order to isolate the enzyme. The extracted material was first tested for the presence of the protein by Lowry's method which showed positive result. The extracted material was now tested for the presence of the enzyme amylase. 1% starch solution was used as substrate in order to test the activity of amylase.

In our first experiment we wished to optimize the amount of the crude extracted enzyme, amylase from the plant material. Here we used different volumes of the extract i.e 1.0ml, 2.5ml, 5.0ml, 7.0ml and 10.0ml [Table 1]. From the observation it could be inferred that the optimum volume of crude extract was 5ml. This is because as we increased the volume above 5ml the activity of the enzymes didn't showed any significant increase. Therefore we used 5ml of crude extract in our further experiments.

We tried to find out the time of incubation for the maximum activity of the enzyme. The time of incubation was varied from 1min to 20 mins. [Table 2].The activity increased from 1min to 10 mins. But we found that from 15mins onwards the amount of starch hydrolyzed started to decrease. However we were not able to cover the short time intervals between 10 mins to 15mins which may show the maximum activity. Therefore, we optimized the incubation period of 10 mins only for our further experiments.

The use of any enzyme for the commercial purpose depends on the temperature in which it shows its maximum activity. Our next aim was to optimize the temperature for the maximum activity of the crude extracted amylase from the foliose lichen. In our experiment a temperature range of 5^{0}C to 55 ^{0}C was maintained [Table 3]. It was observed that at pH 6 and at incubation time of 10 min. the enzyme showed its maximum activity at a very low temperature of 15 ^{0}C. A drastic fall of enzyme activity was recorded as we increase the temperature from 25^{0}C onwards. However, a slight increase in activity was seen at 55^{0}C. This observation can be compared with the occurrence of the foliose lichen that was collected from the hills of Arunachal Pradesh at a low temperature of 10-15 ^{0}C. This low temperature activity of the amylase therefore can be used for the industrial purpose.

For an enzyme activity, the pH is an important factor. So our next aim was to optimize the pH for the enzyme activity. The different pH solutions were used from pH 3.0 to pH 10 [Table 4]. It was found that the enzyme showed maximum activity at pH 7.0 while the activity is less at acidic and basic pH compared the neutral pH.

Further we tried to purify the enzyme amylase by giving the ammonium sulphate cut at different concentrations, which ranged from 1.5M to 4M [Table 5]. It was found that the amylase showed maximum activity at an ammonium sulphate concentration of 1.5 M and 2M. To remove the ammonium sulphate dialysis [Table 6] was done for further purification. It was observed that 0.1ml of the enzyme showed the activity comparable to the crude enzyme extract keeping all the parameters constant such as pH 7.0, incubation period of 10mins and temperature of 15°C.

For the purpose of purification salting out was done by ammonium sulphate. The trace of ammonium sulphate was removed from the protein by dialysis. The activity of the enzyme both in precipitated state in ammonium sulphate and after dialysis were measured and found that the activity has increased significantly [Table 7], with respect to the crude enzyme.

Lastly the partially purified enzyme amylase from the foliose lichen, *Parmelia* was studied to observe the effect of metals on enzyme activity. This was done to see the role of metals as metals behave as the cofactors for the enzyme activity. The different metals used were calcium, potassium, sodium, copper and iron [Table 8]. With Cu, we found that at very low concentration it increased the activity however it inhibited the activity at higher concentration. So, Cu is not desirable in the production process. Ca and Fe, however showed increase in the enzyme activity and are therefore desirable metals for the large scale production of the enzyme. Therefore all these results interpret that the enzyme amylase isolated from the foliose lichen showed its optimum activity at the low temperature of 15°C, at an incubation period of 10mins with Ca and Fe as its metal inducers. This low temperature nature of the enzyme amylase can be exploited in future for its commercial application.

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ARUNACHAL PRADESH: THE NATURAL VIEW