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Screening, Production and Optimization of Protease Enzyme from *Streptomyces* Species

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In the present study Streptomyces species were screened as protease producer Based on the zone of clearance.The enzyme production was optimized using the following parameters like substrate, concentration, different temperature, pH, carbon source and Nitrogen source. The study was done up to 10 days, the crude enzyme was concentrated using Ammoniumsulphate precipitation, dialysis and Lyophilization.The substrate concentration 0.2 of casein was suitable for more protease Production and protein induction. At different pH, the study of pH 7 is favorable for more protease Production 6th day. It is also enhance the protein content According to the temperature study at 30 c of incubation it favor more Protease production and more protein content at 6th day Dextrose (2%) is the best carbon source for the more amount of protease Production and protein content at 10th day Peptone in the most favorable nitrogen source for protease production and protein content. The concentrated enzyme band patterns were observed on SDS-PAGE.

Key words: Protease, Streptomyces, SDS-PAGE, Lyophilization, Precipitation, pH, Dextrose.

Proteases represent the class of enzymes which occupy pivotal Position with respect to their physiological roles as well as their commercial Applications (Moreira et *al.,* 2002). The perform both degratative and synthetic function. Proteases Occur ubiquitously in a wide diversity of sources such as plants, animals and Microorganism. Microbes are and attractive sources of proteases owing to the Limited space required for the cultivation and their ready susceptibility to Genetic manipulation. Proteases are divided into exo and endo peptidases based on their action at or away from the termini, respectively.(Mellouli et *al.*,2003).They are also Classified as serine proteases, aspartic proteases, cysteine proteases and metallo Proteases depending of the nature of the functional group at reactive site. Proteases are the single class of enzyme which occupies a pivotal position with respect to their applications in both physiological and commercial Fields. Proteolytic enzymes catalyze the total hydrolysis of proteins. The current estimated value of the worldwide sales of industrial enzyme is $1Billion of the industrial enzymes. 75% are hydrolytic. Proteases represent one of the three largest groups of industrial enzymes and Account for about 60% of the total worldwide sale of the enzyme. (Chu et *al.*, 2007).They are responsible for the complex processes involved in the Normal physiology of the cell as well as in abnormal pathophysiological Conditions. Their involvement in the life cycle of disease-causing organisms has Led them to become a potential target for developing therapeutic agents fatal diseases such as cancer and AIDS. Proteases are grossly subdivided into two major group, chenoidi Exopeptidases and

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endopeptidases ,depending on their site of action. Exopeptidases cleave the peptide bond proximal to the amino or carboxyl termini of the substrate. Endopeptidases cleave peptide bond distance from the Termini of the sustained (Dastager et *al.*,2008). Based on the functional group present at the active proteases, aspartic proteases, cysteine protease, and metalloprotease Streptomyces are filamentous bacteria consist of vegetative hyphae, produced branched mycelium that rarely fragments. Aerial mycelium at maturity from chains of 3 to many spores. Which are non-motile The colored pigments are diffusible in the medium. The colonies are discrete, lichenoid, leathery or butyrous. on solid medium and produce a Wide variety of pigments responsible for the vegetative and aerial Mycelium (El-Shafei et *al.*,2010).

MATERIALS AND METHODS

Cleaning of Glass Wares

All the glass used in the study was first soaked in Chromic acid. The cleaning solution containing 10% potassium dichromate solution in 25% Sulphuric acid overnight and again washed thoroughly in detergent solution and then washed several times in tap water and finally rinsed in distilled water and oven dried.

Sterilization

Sterilization of all glass wares and various were carried out in an autoclave at 121 c for 15 minutes at 15lbs.

Maintenance of culture

The culture was maintained on sterile starch casein agar slants at 4C.

Preparation of spore suspension

50ml of sterile distilled water added to 100ml flask containing the Culture. The flask was shaken well to harvest the spore from this l ml is taken and inoculated into 50ml of liquid medium(production medium). This method of preparation of inoculum is followed the study.

Incubation

The inoculated flasks were incubated in a rotary shaker 100rpm at Room temperature for about ten days. Every two days the culture filtrate was Harvested and checked for the enzyme activity and total protein content.

Control

50ml of flask of sterilized un inoculated production media was used as blank for protease assay. The following study was done every 2 days of Incubation.

(i) Enzyme activity

(ii) Protein content

Optimisation of protease production

The optimization study of the following parameters was done for Better growth and production of the enzyme.

Effect of different substrate (casein) concentration on protease production 50ml of sterile production medium prepared with different Substrate (casein) concentration of 0.1,0.2,and 0.3, 0.4 and 0.5% then all conical Flask inoculated with l ml of spore suspension of the culture and incubated under the shaking condition (100rpm). Then culture filtrate was harvested for Every 2 days. The enzyme assay and protein content was studied up to 10 days.

Sterile un inoculated production medium was used as blank for protease assay. Effect of different pH on protease production 50ml of production medium was prepared with pH of the medium was adjusted to 5.5,6.0,6.5,7.0,7.5 and 8.0. The sterilized production medium was inoculated with 1 ml of spore suspension and incubated under the shaking condition. The enzyme activity and protein content under the shaking condition and study was done for every two day up to 10 days .Sterile un inoculated Production medium was used as blank for protease assay. Effect of different temperature on protease production 50ml of production medium was prepared and sterilized and 1 ml Inoculum was added then incubated at different temperatures of 20 C, 25 C, 30C, 35 C and 40 C , The culture filtrate was harvested of every two days. The enzyme activity and protein content was studied for up to 10 days. effect of different carbon sources on protease production 50 ml of production medium with different carbon sources (2%)Such as dextrose, maltose, lactose, fructose and galactose was prepared , sterilized, inoculated and incubated up to 10 days under the shaking Condition. The culture filtrate was harvested for every two days. The enzyme activity and protein content were studied.50 ml of production medium amended with different organic nitrogen sources (0.1%) like peptone, beef extract, yeast extract, gelatin and tryptone was prepared and sterilized. 1 ml of spore suspension was inoculated and

incubated under the shaking condition up to 10 days. Culture filtrate was harvested and studied for enzyme activity and protein content for every two days.

Estimatiom of crude enzyme from production medium

The culture filtrate was harvested from production medium at every two days of time interval up to 10days . The protease activity and protein content were studied.

RESULTS AND DISCUSSION

Effect of different concentration of substrate on protease production and Protein content the production medium containing different substrate concentration(Casein) like 0.1%, 0.2%, 0.3%, 0.4%, 0.5% were tested for protease production at different days of incubation period. The table. 1 and fig. 1 shows maximum protease production was observed in 0.2% substrate concentration at the 6th day (710 U/ml). Followed by in 0.1%of the substrate concentration (600U/ml) of protease production was observed at 6th day . After incubation enzyme production was gradually decreased. Maximum amount of protein content (600ug/ml) was observed in 0.2%Substrate concentration at 6th day, followed by 0.1% of substrate concentration(520ug/ml) of protein content at 6th day.

Effect of different pH on protease production and protein content of Streptomyces sp. effect of different pH on protease production and protein content. The effect of different pH on protease production and protein content was summarized in table.2 and fig.2.Amount the different pH checked maximum enzyme production (780U/ml) was observed at 6th day at pH 7. The pH 7 produce (360U/ml) of Protease at 10th day. After incubation the enzyme production gradually decreased. The protein estimation showed that the maximum protein content Observed (320 ug/ml) at 8th day.

Effect of different temperature on protease production and protein Content the production medium inoculated with 2% inoculum incubated under the shaking condition at 20 C,25 C,30 C,35 C and 40 C up to 10 days. The result was

Table 1. Effect of different substrate concentration on protease on production and protein content of Streptomyces species

Substrate Concentration	Enzyme Activity (U/ml) Days of Incubation					Protein Content (ug/ml) Days of incubation				
	2nd day	4th day	6th day	8th day	10th day	2nd day	4th day	6th day	8th day	10th day
0.10%	200	379	600	330	250	60	80	520	30	30
0.20%	420	599	710	520	370	120	380	600	40	80
0.30%	150	259	590	360	240	70	163	310	150	110
0.40%	80	195	320	160	120	15	72	120	230	10
0.50%	60	162	290	200	80	23	49	162	360	220

Table 2. Effect of different pH on protease production and protein content of Streptomyces sp

Different pH	Enzyme Activity (U/ml) Days of Incubation					Protein Content (ug/ml) Days of incubation				
	2nd day	4th day	6th day	8th day	10th day	2nd day	4th day	6th day	8th day	10th day
5.5	220	340	100	150	90	80	190	180	120	100
6	180	370	230	270	180	50	140	110	110	150
6.55	160	240	275	350	290	70	210	150	150	110
7	210	360	780	230	360	110	140	120	160	410
7.5	250	30	520	720	480	150	250	130	180	120
8	100	80	320	20	10	220	290	210	320	170

present in table.3. The maximum enzyme production was observed at 30 C at 6th day (790U/ml. The poor enzyme production observed At 20 C at 2th day 45/ml)the maximum protein content observed at 30 C at 6thday(540ug/ml).

Effect of different carbon sources on production and protein content the production medium containing different carbon sources (2%) i.e., Lactose, Dextrose, Fructose, Galactose, Ribose and Maltose after incubation they incubated up to 10 days under the shaking conditions. Results presented in table. 4 and fig.4 showed that the maximum protease production (1050U/ml) where 2%dextrose is used as carbon source at 10th day. Followed by Lactose produce (365 U/ml) at 6th day. Galactose produced very Low amount of enzyme (6U/ml) at 10th day the protein estimation shows that maximum protein released in Ribose(510ug/ml) at 4th day. Followed by Maltose produced (440ug/ml) of protein at 6th day.

Effect of different Nitrogen sources on protease on protease production and protein content The production medium supplemented with different nitrogen sources like Beef extract, Tryptone, Yeast extract, Peptone and Gelatin (0,1%)were tested for protease production at

Table 3. Effect of different temperature on protease production and protein Content of Streptomyces sp

Different Temperature	Enzyme Activity (U/ml) Days of Incubation					Protein Content (ug/ml) Days of incubation				
	2nd day	4th day	6th day	8th day	10th day	2nd day	4th day	6th day	8th day	10th day
20C%	45	90	270	190	160	15	22	80	120	60
25C%	80	150	370	290	252	28	42	200	340	270
30C%	120	227	790	670	600	75	148	540	462	400
35C%	102	179	640	649	500	19	35	275	290	175
40C%	35	82	250	275	200	24	65	175	82	63

Table 4. Effect of different carbon source on production and protein Content of Streptomyces sp

Different Carbon Sources	Enzyme Activity (U/ml) Days of Incubation					Protein Content (ug/ml) Days of incubation				
	2nd day	4th day	6th day	8th day	10th day	2nd day	4th day	6th day	8th day	10th day
Lactose	80	240	365	110	89	5	20	210	180	120
Dextrose	110	480	830	800	1050	2	30	160	60	110
Fructose	20	8	48	30	130	1	120	210	30	6
Galactose	10	23	30	10	6	5	50	150	10	7
Ribose	20	42	80	60	47	200	510	160	9	180
Maltose	50	70	98	330	50	20	8	440	60	4

Table 5. Effect of different nitrogen source on protease production and protein Content of Streptomyces sp

Different Carbon Sources	Enzyme Activity (U/ml) Days of Incubation					Protein Content (ug/ml) Days of incubation				
	2nd day	4th day	6th day	8th day	10th day	2nd day	4th day	6th day	8th day	10th day
Beef Extract	610	220	20	558	340	9	10	10	30	80
Tryptone	150	160	580	900	400	20	30	10	40	80
Yeast Extract	258	370	790	920	440	10	30	20	10	80
Peptone	170	360	820	1010	610	10	40	30	40	60
Gelatin	110	410	690	930	360	50	80	8	60	40

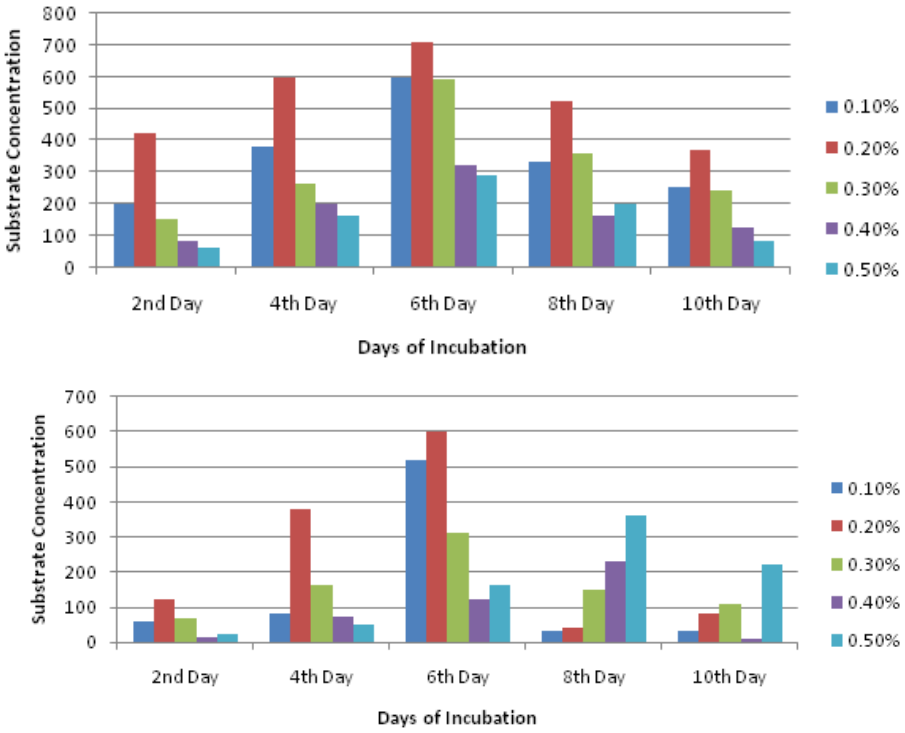


Fig. 1. Effect of different substrate concentration on protease production and Protein content of Streptomyces sp.

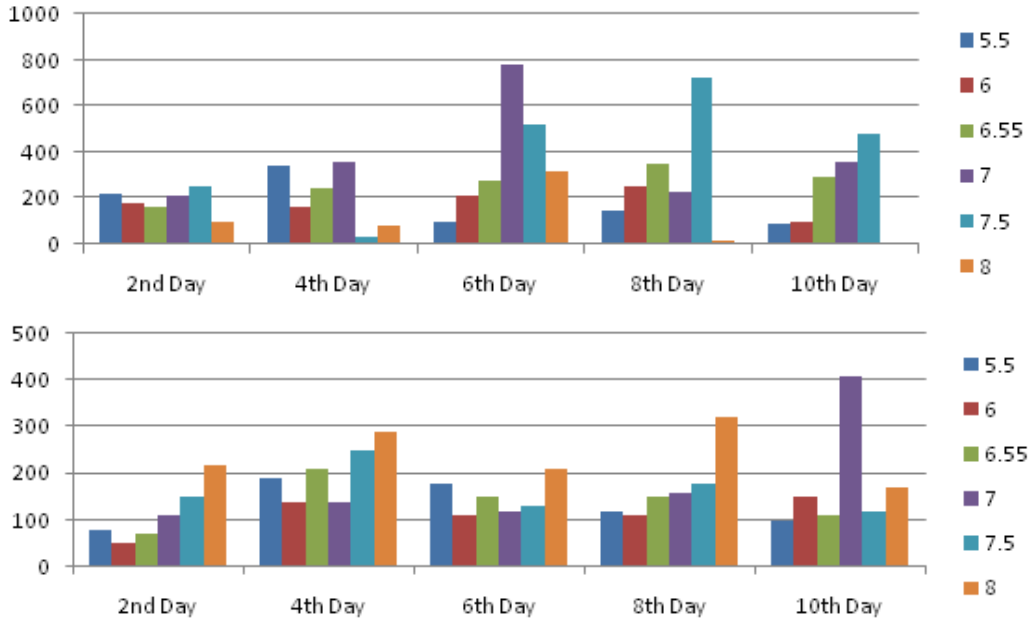


Fig. 2. Effect of different pH on protease production and protein content of Streptomyces sp. effect of different pH on protease production and protein content. The effect of different pH on protease production and protein content was summarized in table.2 and fig.2.Amount the different pH checked maximum enzyme production (780U/ml) was observed at 6th day at pH 7. The pH 7 produce (360U/ml) of Protease at 10th day. After incubation the enzyme production gradually decreased. The protein estimation showed that the maximum protein content Observed (320 ug/ml) at 8th day.

different days of incubation. The table.5 andFig.5, shows maximum protease production was observed in peptone(1010U/ml) at 8th day. Followed by Gelatin produce (930U/ml) at 8thday. The protein estimation shows that The maximum protein released in Beef Extract (80ug/ml) at 10th day. Followed by peptone produced (60ug/ml).

The 1000ml of culture filtrate treated with

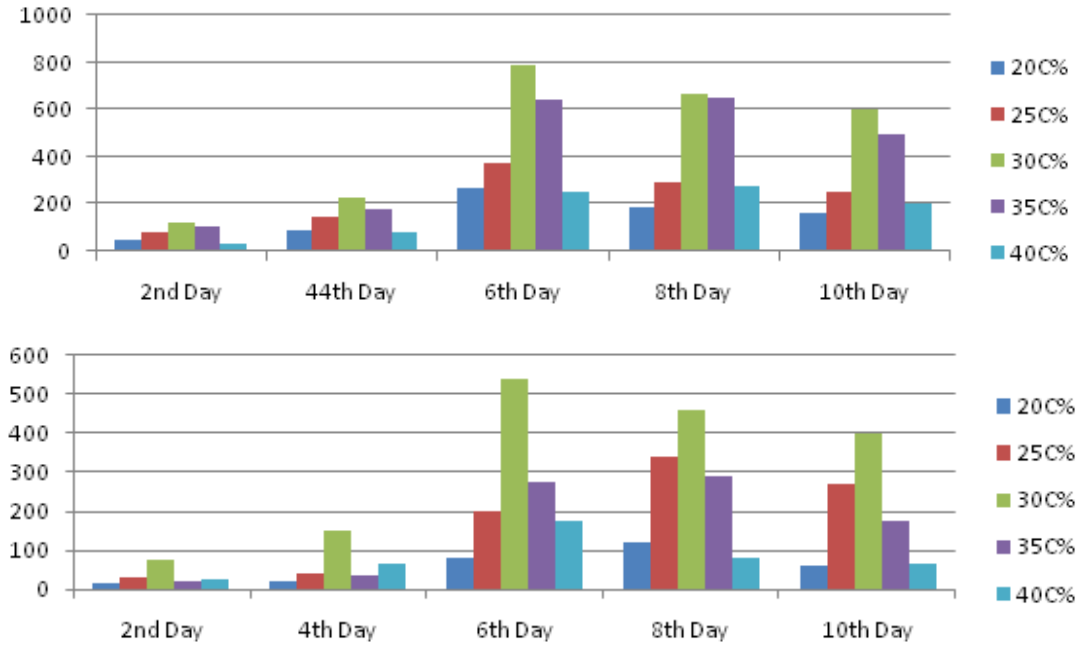


Fig.3. Effect of different temperature on protease production and protein Content of Streptomyces sp

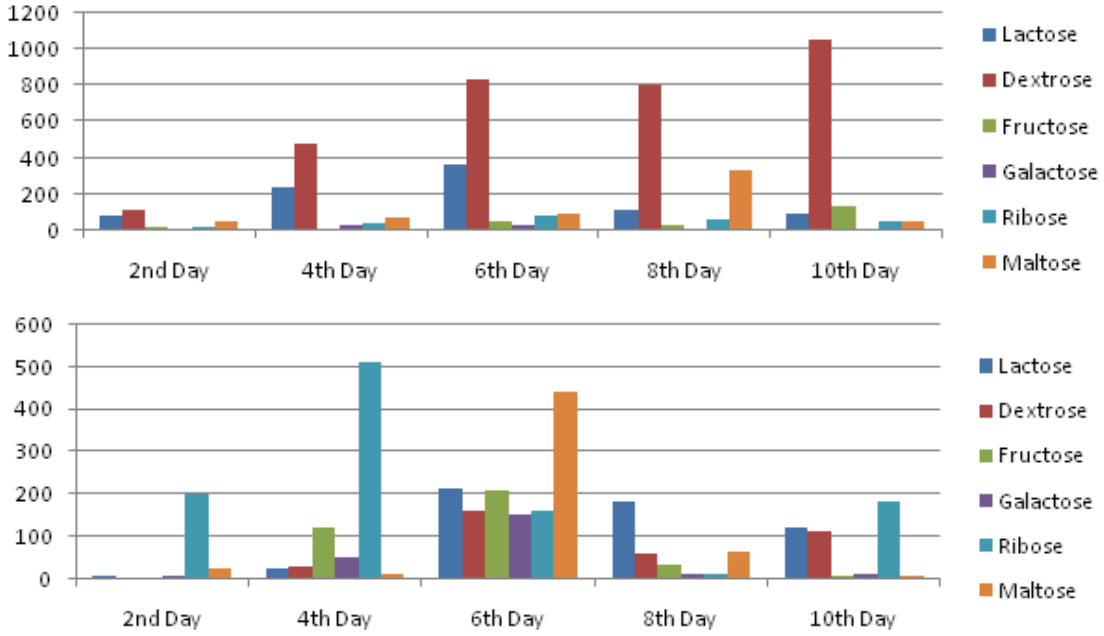


Fig. 4. Effect of different carbon source on production and protein Content of Streptomycessp

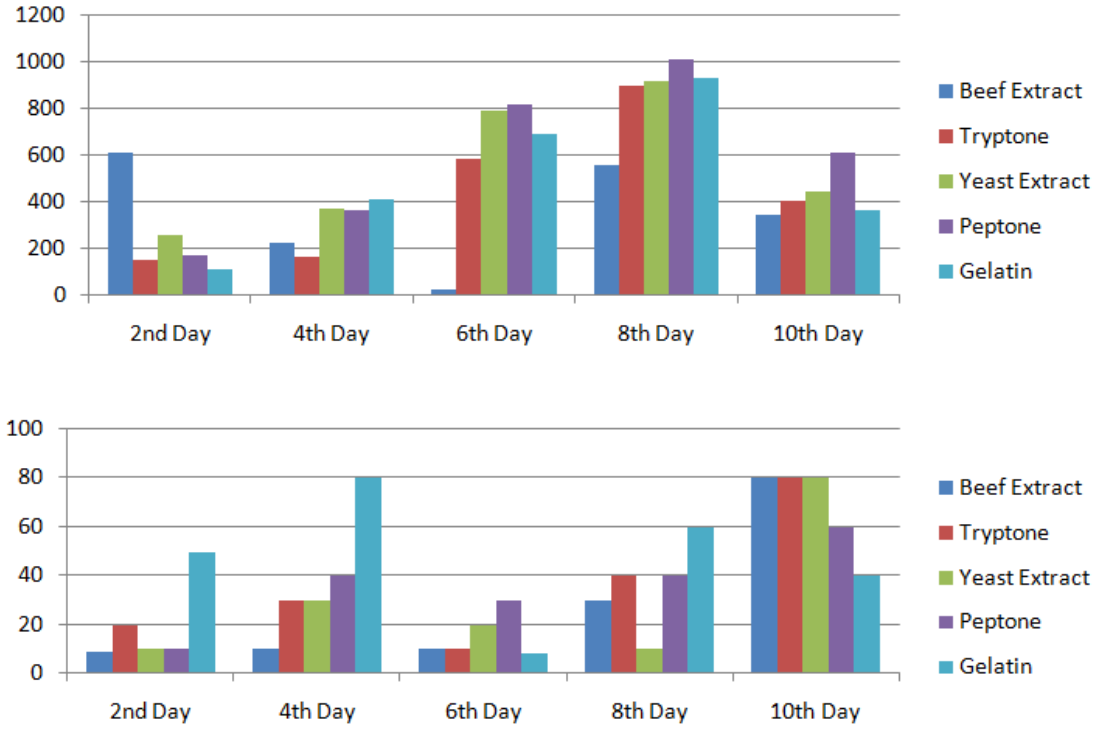
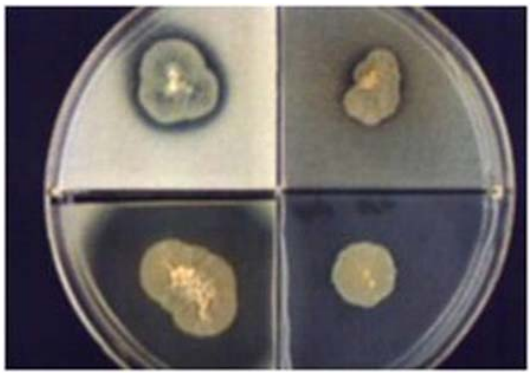
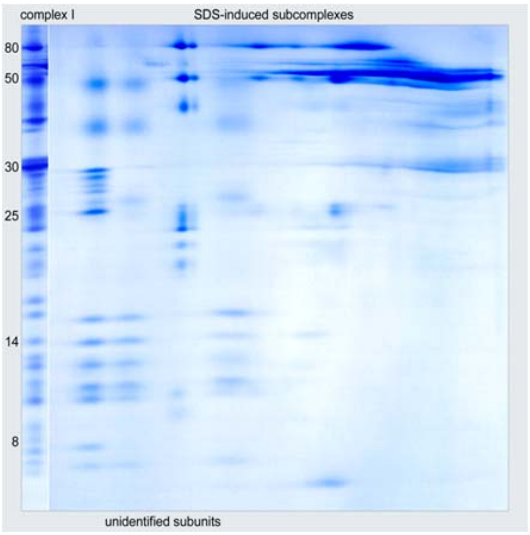


Fig. 5. Effect of different nitrogen source on protease production and protein Content of Streptomyces sp

Streptomyces sp growth on starch casein agar medium



Ammonium Sulphate precipitation



Protein band patterns of Streptomyces sp crude culture filtrate on SDS-PAGE

70% of ammonium sulphate Saturation. The crude enzyme was continuously stirred using magnetic stirrer and kept 4 at over night, the enzyme was centrifuged at 10,000 rpm for 15 minutes,and And Then supernatant was discarded. The pellets were collected using(Glass distilled water) Dialysis Pretreatment of dialyses membrane

Dialysis membrane was treated in water bath at 60 C for 20 minutes.Then it was washed thoroughly with glass distilled water. A knot was made at One end the membrane and in used for filling the sample. to this dialyses bag, sample was added, it was dialysed against glass

Distilled water for overnight at 4' by placing on a magnetic stirrer. The buffer used for dialysis should be removed twice for the complete removal of salts.

Lyophilization

The dialysed sample was collected in a sterile round bottom flask. It was Rotated on ice at 45' angle for the formation of uniform thin layer coating of sample on the flask and lyophilized using the virtis lypophilizer. The concentrated enzyme band patterns were observed on SDS-PAGE.

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