Isolation, Characterization And Optimization Of Amylase Producing Organisms From Soil of Sabarkantha District

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Abstract *- Amylases are amongst most widely used enzymes in industries such as food, fermentation, starch processing, textile and paper. In the present investigation, bacteria were isolated from Garden a farm site of different regions of the Idar Sabarkantha District, screened for the production of amylase and their optimum growth conditions were determined. A total 10 bacterial colonies were isolated from collecting soil samples. Ten bacterial isolates, displayed zones of clearance in the starch hydrolysis test. The isolate displaying maximum amylase activity on the constitution was selected. A characteristic feature of the strain indicates that it belongs to the genus Bacillus and streptomycin and will be later used for further characterization. Maximum yield of amylase was obtained after 48hrs of incubation. The optimum pH, carbon source, Nitrogern Source,Salt, Temperature,Shaking seed and Growth Factor for enzyme activity.*

Keywords: *Bacillus,streptomycin, starch hydrolysis,carbon source,Nitrogen Source, optimum temperature*

I. INTRODUCTION

The enzymes from microbial sources are more stable and obtained cheaply. Among them Amylases are the most important enzymes and are of great significance in the present day industry.(Alariya et al., 2013). Soil enzymes are central to ecosystem processes because they catalyze innumerable reactions in soils that have biochemical significance. (Dick & Kandeler, 2005).Microbial enzymes have been generally favored for their easier isolation in high amounts, low-cost production in a short time, and stability at various extreme conditions, and their compounds are also more controllable and less harmful (Gopinath et al., 2017). Were Starch degrading bacteria are most important for industries such as food, fermentation, textile and paper.(Singh & Kumari, 2016) Also play a wider role in biochemical cycle of carbon and also has a wider application in the biotechnologicl base product. Many researchers have studied amylase production with a variety of substrates and microorganisms such as bacteria, yeast and fungi in a wide range of applications of amylase enzymes in various sectors such as confectionery, baking, paper, textiles, detergents and many pharmaceuticals. (Bala et al., 2013) Simillarly the Soil fertility has a direct relation with the crop yields, provided other factors are in optimum level. Which periodically estimated as there is continuous removal of nutrients by the crop intensively grown in every crop season (Patel at el., 2017). The pH and availability of essential plant nutrients in the soil must also be known. to evaluate the soil's physical, chemical and microbiological properties, to determine whether these soil parameters can provide a good evaluation of the performance and sustainability of the agricultural and forestry system. (Chopra, 2006). Amylase is a hydrolytic enzyme and in recent years, interest in its microbial production has increased dramatically due to its wide spread use in food, textile, baking and detergent industries. .(Singh & Kumari, 2016) amylase activity has been synthesized by coupling Procion Yellow dye with starch. and is easily suspended in water, in neutral buffer solution, and also in acidic solution. Amylase from pancreatin, saliva, urine and serum readily hydrolyzes this chromogenic substrate.(Jung, 1980).

II. Materials and Methods

Isolation of Amylase Producing Microorganisms:Soil samples were collected from Garden an farm sites from idar . Serial dilution was made and was plated on nutrient agar by spreading 0.1ml of the diluted sample. Then the plates were kept for incubation at 37°C for overnight.Macroscopic & Microscopic observation was carried.

Screening of potential amylase producing bacteria by starch hydrolysis test :Bacterial isolates were screened for amylolytic activity by starch hydrolysis test on starch agar plate.The microbial isolates were streaked on the starch agar plate and incubated at 37°C for 48 hours. After incubation iodine solution was flooded with dropper for 30 seconds on the starch agar plate.The Presence of blue colour around the growth indicates negative result and a clear zone of hydrolysis around the growth indicates positive result. The isolates produced clear zones of hydrolysis were considered as amylase producers and were further investigated.(Gebreyohannes, 2015)

Morphological and Biochemical Characteristics:Gram staining Sugar fermentation,Fructose,Dextrose,Mannitol,Sucrose,Xylose,Lactose,Catalase test,MR Test,VP Test,H2S Prod.,Ammonium Prod.,Casin Hydrolysis,Citrate Utilization,Nitrate Reduction,Motility Test were carried out.

Quantitative test for amylase enzyme

Production Of Amylase Enzyme

Preparation of seed culture: Bacterial isolates from their respective slants were inoculated in 100 mL nutrient broth under aseptic condition. Flasks were placed in a shaker at 37oC, 120 rpm for 24hrs

Enzyme production medium: Production medium contained (g/l) Bacteriological peptone 6.0gm, MgSO4.7H20 0.5gm, KCL 0.5gm, Starch 10gm. 10 ml of medium was taken in a 100 ml conical flask. The flasks were sterilized in autoclave at 1210C for 15 min and after cooling the flask was inoculated with overnight grown bacterial culture. The inoculated medium was incubated at 370C in a shaker incubator for 24hr. At the end of the fermentation period, the culture medium was centrifuged at 5000 rpm for 15 min to obtain the crude extract, which served as enzyme source.

Extraction of enzyme from **bacterial fermentation broth:** At theend of the fermentation period enzyme analysis at 24, 48, 72 hrs. Bacterial fermentation broth was taken into a centrifuge tube and it was centrifuged at 5000 rpm for 10 min. After centrifugation, the bacterial cell pellet was removed and bacteria free supernatant was used as a source of crude enzyme.

Determination of Amylase Activity: Amylase activity in the crude enzyme extract was determined by estimation of reducing sugar (maltose) liberated by the action of amylase on soluble starch. Reducing sugars were estimated by dinitrosalicylic acid (DNS) method of (Vaikundamoorthy et., al, 2018) (Manimaran & Kannabiran, 2018).Amylase Assay The enzyme activity was assayed following the method of Bernfeld (1955) using 3, 5- dinitrosalicylic acid..

Process Optimization for Amylase Production

Carbon Source: Various carbon source such as dextrose, maltose sucrose, fructose were used to study the effect on amylase production. These carbon sources were individually added in the production medium at a constant concentration. The carbon source of original production medium was replaced by alternative carbon sources (Alariyaet al., 2013).

Organic and Inorganic Nitrogen Sources:Various nitrogen sources like yeast extract, beef extract, ammonium sulphatewere used to investigate effects on amylase production. These nitrogen sources were added individual in the fermentation broth at a constant rate. The enzyme assay carried out after 24 hrs of incubation.

Cation Salt: Various cation salt like cobalt chloride, calcium chloride and manganese chloride were used to check the effect on amylase production. The enzyme assay carried out after 24 hrs incubation

pH: Fermentation broth for each isolates adjusted with different pH range 6, 7, 8 using 1N HCl and 1N NaOH. Inoculated and incubated at 370C, 120 rpm for 24 hrs.Carriedout enzymes assay after the incubation period.

Temperature: Fermentation broth for each isolate was inoculated. These medium flasks were incubated at different temperatures like 300C, 400C, 450C, 450C at 120 rpm for 24 hrs incubation. After incubation carried out enzyme assay.

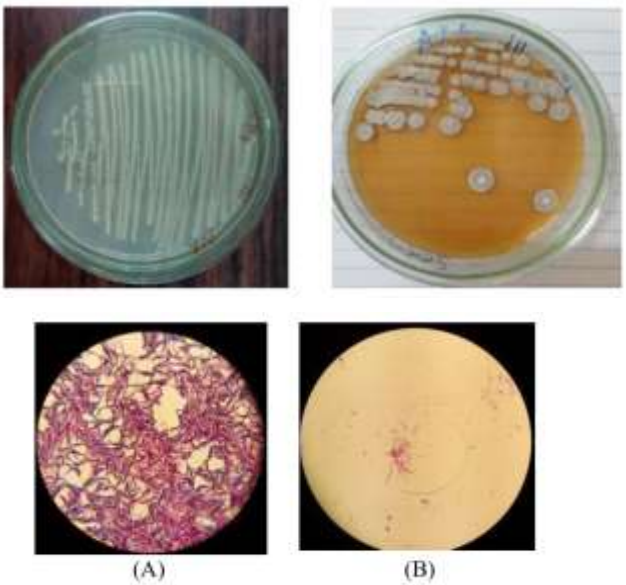
Shaking Speed: Fermentation broth inoculated with active culture and then placed in shaker incubator with different shaking speed like 120, 150, 170 rpm for 24 hrs incubation. Carried out enzymes assay after the incubation period

Growth Factor: Fermentation broth has added 0.0005 gm/100mL biotin as growth factor to check the effect on amylase production. Carried out enzymes assay after the incubation period

3 Results And Discussion

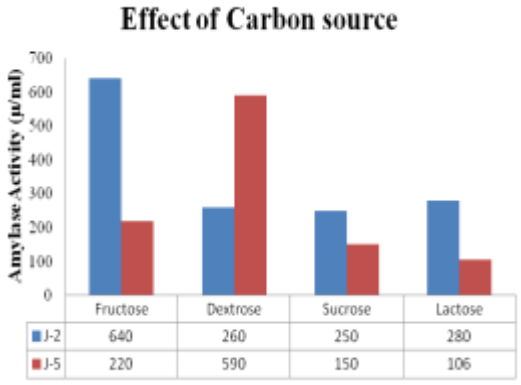
Screening of Amylase Producing Bacteria: The bacteria isolated from soil were screened for amylase production on starch agar medium. From soil samples 10 bacterial strains were isolated. Among them, it was found that only 2 strains showed highest amylase activity zone in Starch agar plate .further optimization was carried on two isolates.

Sr. no	Code	Zone	Sr. no	Code	Zone
1	J1	5 mm	6	J6	2 mm
2	**J2**	**6mm**	7	J7	1 mm
3	J3	3 mm	8	J8	4 mm
4	J4	4 mm	9	J9	0.3 mm
5	**J5**	**11 mm**	10	J10	0.6 mm

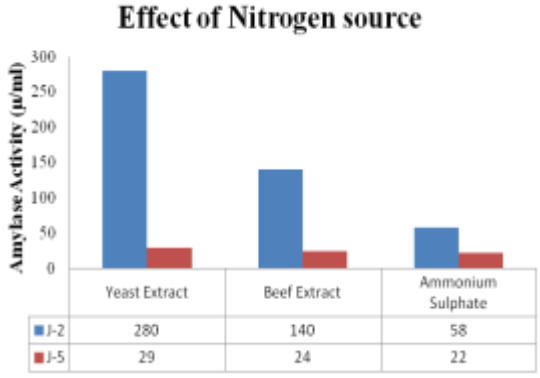


(A) (B)

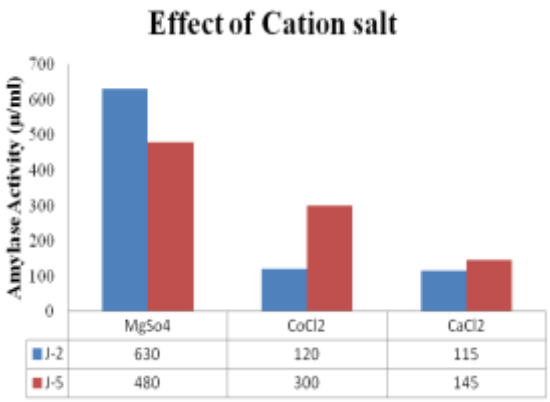
Carbon source: Various sources of Carbon such as Dextrose, Fructose, Lactose and Sucrose were used to replace Starch which was the original carbon source in growth media. Results obtained showed that, fructose brought the highest amylase production compared to other carbon sources at 24 hour incubation in (Viswanathan et.,al ,2014) reported that the different carbon sources have varied influences on the extracellular enzymes especially amylase strains. Results obtained showed that increase in concentration of various substrates increases the amylase strain .Fig-1.



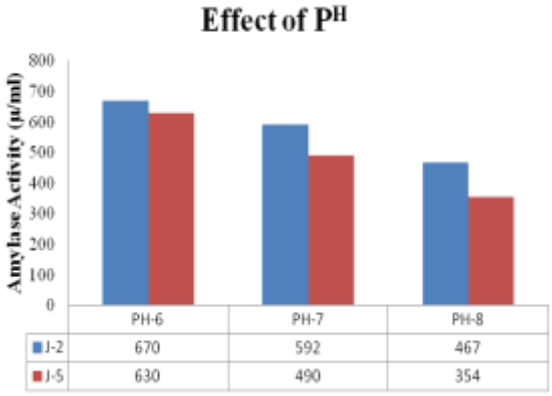
Nitrogen Sources: Various sources of nitrogen such as yeast extract, beef extract and ammonium sulfate were used to replace peptone which was the original nitrogen source in growth media. Results obtained showed that Yeast extract brought the highest amylase activity 280 µ/ml in J2 sample and 29 U/ml in J5 sample compared to other nitrogen source at 24 hrs incubation in both bacterial strains.(Aqeel & Umar,2010)



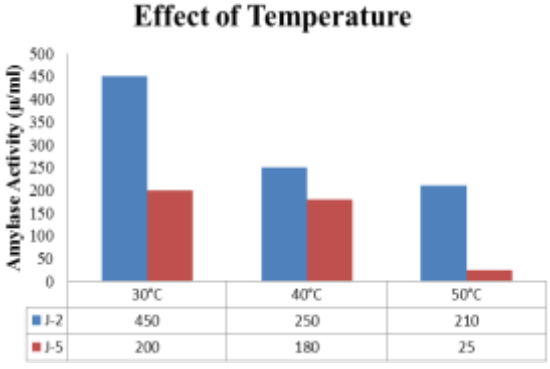
Effect of cation salt: Various cation salts such as Magnesium sulfate , cobalt chloride and calcium chloride was used as cation salts in growth media. Results obtained showed that Magnesium sulfate brought the highest amylase activity 630 µ/ml in J2 sample and 480 µ /ml in J5 sample compared to other cation salt at 24 hrs incubation in both strains.



Effect of pH: the pH 6, 7, 8 different pH of fermentation medium was tested for the amylase production. Both isolates were allowed to grow in these three pH range. Maximum amylase activity was observed in medium of pH 6.0 in both strains . J2 sample observed 670 µ/ml and J5 sample has observed 630 µ/ml amylase activity at 24 hrs incubation.

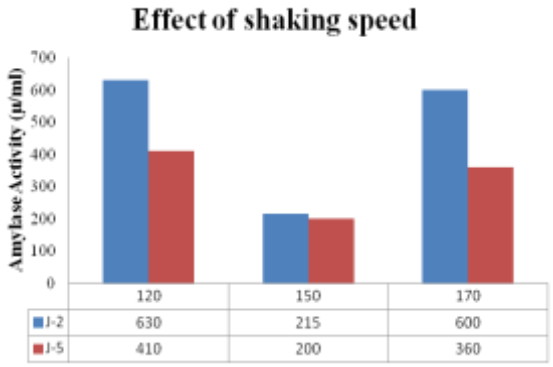


Effect of temperature: Enzyme activity at different temperature ranging from 30 .C , 40 .C, 45 .C. Maximum amylase activity was observed in temperature of 35 .C and then activity are gradually reduced at 24 hrs incubation.

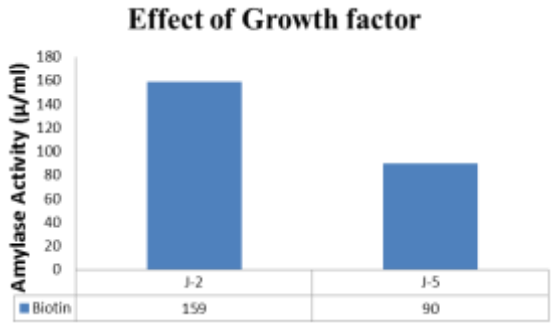


Effect of shaking speed: The amylase activity tested at different shaking speed was 120 rpm, 150 rpm and 170 rpm .The maximum amylase activity was

observed at 120 rpm in both strain compared to the other, has observed 630 µ/ml and J5 has observed 410 µ/ml amylase activity.



***The Effect of growth factor*:** In fermentation medium 0.0005 gm biotin was added as growth factor for tested the amylase activity. In both strains J2 sample were observed amylase activity was 167 U/ml and J5 sample observed 90 U/ml amylase activity after 24 hrs incubation.



Molecular analysis : Form identification of the isolates, it was subjected to 16 rRNA analysis and on the basis of 16SrRNA, the isolate was identified as Bacillus megaterium and Streptomyces spp.the sequence analysis and its accession number are depicted in the table. The sequence showed 100% similarity with Bacillus megaterium and 96 % similarity with Streptomyces spp.

Sample Code	Sequence	% Similarity	Organism	Accession no.
J2	ACTTGC..... .....AAATGT	100	*Bacillus megaterium*	JDMH01000006.1
J5	CCAAACC..... ..... TAAATA	96	*Streptomyces spp*	KY236015.1

Conclusion

Total of 10 bacterial strain was isolated from farm and garden soil. Soil sample was collected from sabarkantha district. The morphological, biochemical and 16SrRNA considered that J2 sample was identified as *Bacillus* spp. and J5 sample was identified as *Streptomyces* spp. Both strain give highest amylase activity. *Bacillus megaterium* gave maximum enzyme activity in the presence of fructose as carbon source , yeast extract as nitrogen source , magnesium sulfate as cation salt , pH 6, 35º C tem , 120 rpm. *Streptomyces* spp. Gave maximum enzyme activity in the

presence of dextrose as carbon source, beef extract as nitrogen source, magnesium sulfate as cation salt, 6pH, 35ºC, 120 rpm.

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