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Optimization of Culture Conditions for Amylase Production from Aspergillus niger

Motwani D. R.[1], Meshram R. L[1]and Chandak A. M.[2]
1 DRB SindhuMahavidyalaya, Department of Biochemistry, Panchpaoli,Nagpur.440017,Maharashtra,India.
2 DRBSindhuMahavidyalaya, Department of Microbiology,
Panchpaoli, Nagpur 440017,Maharashtra,India.

*****Correspondence: Dr. D.R Motwani, Assistant Professor , Deptt. of Biochemistry,
DRB SindhuMahavidyalaya, KTR Marg, Panchpaoli Nagpur 440017 Maharashtra (India)
Email:daminimotwani7@gmail.com

Abstract

Micro-organisms are capable of producing extracellular enzymes such as Amylases ,pectinases etc. having vast industrial applications. A large number of micro-organisms including bacteria, yeast and fungi produce different groups of enzymes. The main objective of the study was to isolate,produce and optimize Amylase enzyme using *Aspergillus niger* isolated from fruit peel soil waste. The fungal strain was regularely subcultured on Potato dextrose agar. The isolate was further characterized based on colony morphology by performing cotton blue staining and microscopic mount and confirmed as *Aspergillus niger* The fungi *Aspergillus niger* was found to be as Amylase producer by growing on starch agar media. The *Aspergillus niger* was potent producer of enzyme Amylase showed highest activity at pH8.0,temperature 40˚C and incubation period of 6 days. Effect of different Agrowaste on Amylase production proved that wheat bran act as inducer of enzyme Amylase.

Key words:Amylase, *Aspergillus niger*,Starch, Agrowaste and Wheat bran.

I INTRODUCTION

In the modern era Amylase enzyme has received a great deal of attention because of their various applications in food , sugar , textile , leather , pharmaceutical , paper and detergent industries . The literature says that Amylase accounts approximately for 25 % of enzyme market by Dabai et al [3]. The microorganism such as bacteria , fungi and yeast are considered as potential

producers of Amylase enzyme.The benefits of using microorganisms for production of enzyme is that largescale production is economical and microbes are easy to manipulate by Shah et al[8]. Fungal enzyme are preferred over other microbial sources since they are widely accepted Generally Regarded As Safe (GRAS) status by Sindhu R et al[9].

Amylase is extracellular enzyme that hydrolyse α-D-(1,4) glycosidic bonds in starch components and related polysaccharides to release maltose and disaccharide . The production of Amylase is dependent on the strains method of cultivation , incubation period , carbon source , nitrogen source pH, metal ions and thermostability . Many different species of fungi inhibits the soil surface where aerobic conditions exist , such fungi are active in degrading a wide variety of biological materials present in soil by Saranraj and Stella [7]. Studies on fungal Amylase enzyme have concentrated mainly on *Aspergillus species* due to there ubiquitous nature and less nutritional requirements by Abe.J.Bergenum[1]

II MATERIALS AND METHODS

Collection of soil samples

Soil sample was collected from fruit peel waste under sterile condition to avoid contamination and transferred to laboratory using sterile polythene bags .

Isolation of fungal isolate

Asuspensionof soil sample and sterile distilled water was prepared , and plated on potato dextrose agar by Mukunda et al[6] . A broad spectrum antibiotic chloramphenicol , was use to inhibit bacterial growth . The plates were incubated at 28˚C for 2- 3 days .

Microscopic identification of fungal isolates

Fungal species were identify as per the manuals of Domsch et al[4]. The fungal species were subjected to lactophenol cotton blue staning and then analysed for morphology under required magnification .

Isolation of amylolytic fungal isolate

The amylolytic fungi were screened by growing on starch agar medium . Starch agar was prepared by method of Ugoh and Ljigbade [12] The fungus *Aspergillusniger* gave maximum clear zone and was further selected as potential single strain for Amylase production.

Production of Amylase of Aspergillus niger

Fermentation was carried out using *Aspergillusniger* for Amylase production . The fermentation medium contains KH_2PO_4-0.14g,NH_4NO_3-1g, KCl-0.5g,$MgSO_4.7H_2O$-0.01g,$FeSO_4.7H_2O$-

0.001g,soluble starch-2g,Distilled water-100ml at pH6.5.The medium was autoclave at a 121˚C for 20 minutes . Spore suspension(1ml) was added and incubated at 28˚C for 2 to 3 days .

Extraction of Amylase enzyme

The fermentated medium was centrifuged and cell freesupernatend was used for estimation of Amylase activity .

Amylase assay

Amylase activity was estimated by Miller method [5]. The absorbance was measured at 540 nm by photometric colorimeter . A standared graph for Amylase enzyme was prepared .One unit (U) of Amylase activity was described as the amount of enzyme that released µmol of reducing sugar per minute, under the assay conditions.

III RESULTS AND DISCUSSIONS

Optimization of culture conditions

Effect of incubation period on Amylase production

Optimization of incubation period is an essential parameter for maximum growth of *Aspergillus niger* and hence greatly affects Amylase production.The results as shown in the Fig.1 revealed that incubation period 6 th day was found to be best for amylase activity . Amylase activity decreased as the incubation period increased from 6 thday onward .Avalibility of nutrients and moisture in the medium contributes to the growth of *Aspergillus niger* . The result is similar to those reported by shah et al,2014.for*Aspergillus species* .

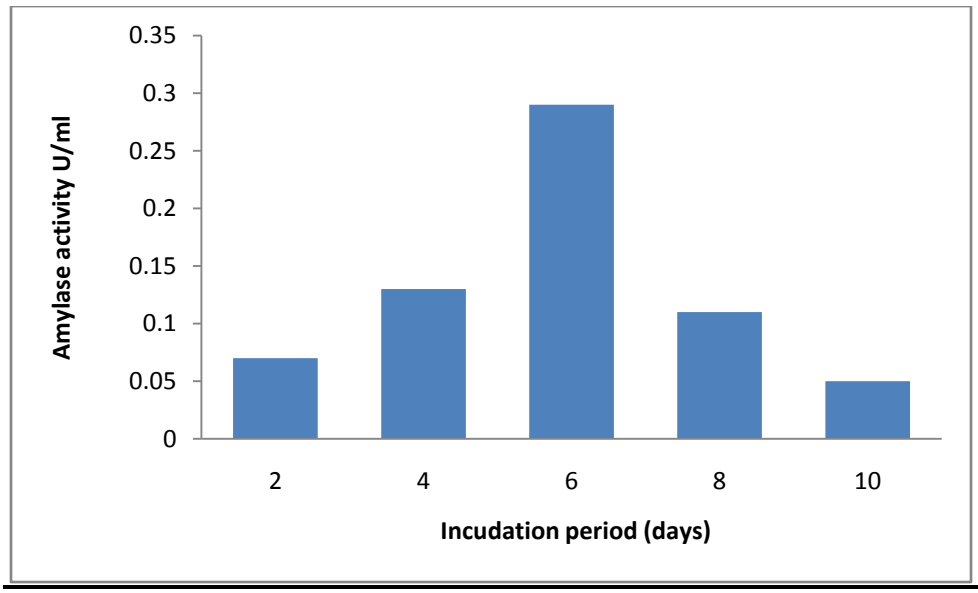

Fig.1:Effect of incubation period on Amylase production

Effect of Temperature on Amylase production

Incubation of fermentation medium at various temperature was performed . Fig :2 shows that maximum Amylase production was observed at 40˚ C by *Aspergillusniger* . Temperature is a critical factor which markedly influence Amylase production .Amylase production was low at 25˚ C and goes on increasing as temperature increases to 40˚ C and then there was a decreased in Amylase activity .

Similarly Spier et al,2006 reported that 45˚ C was optimum for Amylase activity by *Aspergillus species* , however Suganthi et al[11] and Ugohand ljigbadeet al[12] reported that temperatures 30,37 and 40˚ C was optimum for Amylase activity by *Aspergillus species* .

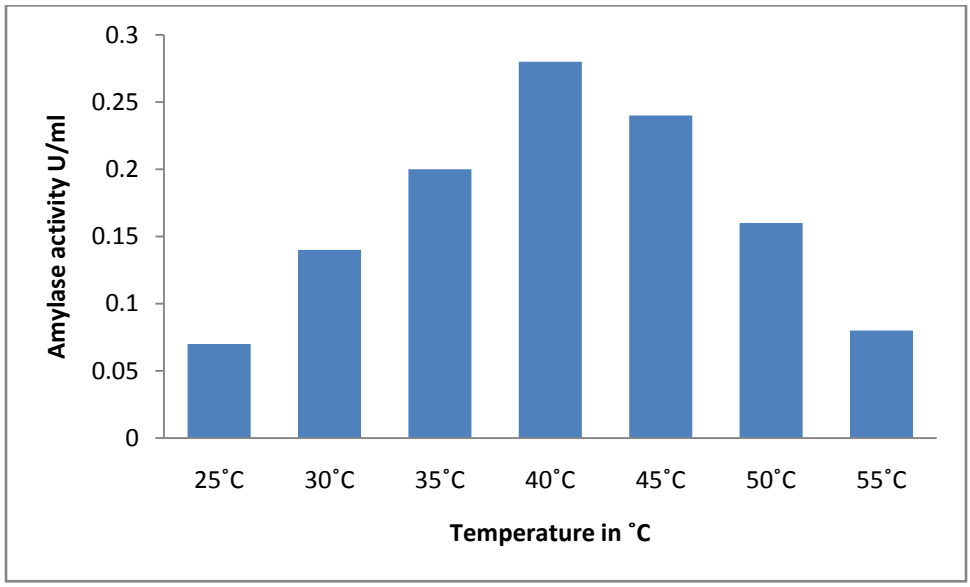

Fig.2:Effect of Temperature on Amylase production

Effect of pH on Amylase production

The effect of pH was studied by varying the pH of the medium from 2.0 to 14.0. pH affects the catalysis activity of Amylase enzyme Shah et al[8]Fig :3 shows that maximum Amylase activity was observed at pH 8.0 . pH changes the metabolic activity of Amylase producing strain .

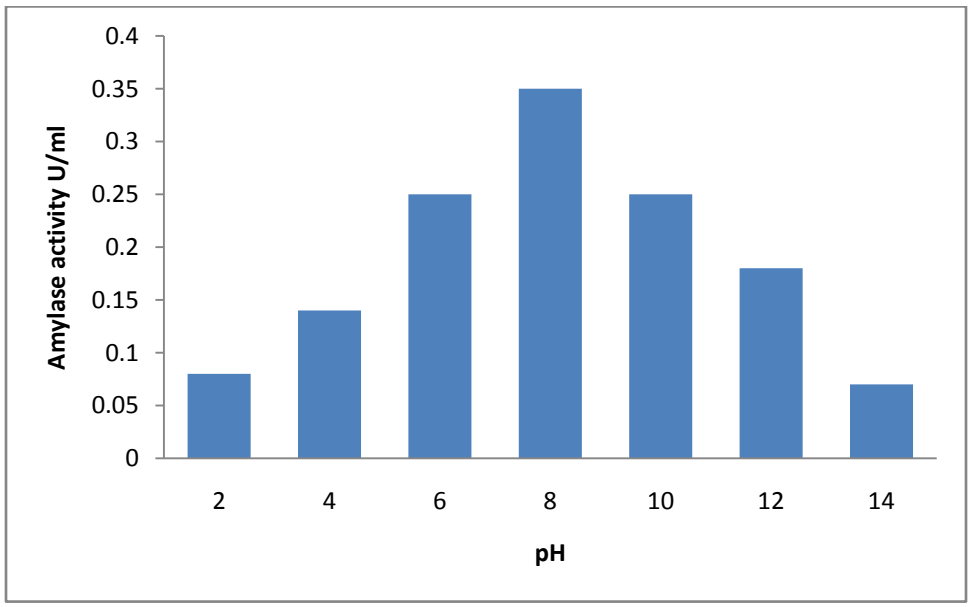

Fig.3:Effect of pH on Amylase production

Effect of Agrowaste on Amylase production

The effect of different agro based waste materials on Amylase enzyme production was investigated by adding soya cake , rice bran , wheat bran and coconut cake . Fig: 4 explains that Agro waste wheat bran gave maximum Amylase production .

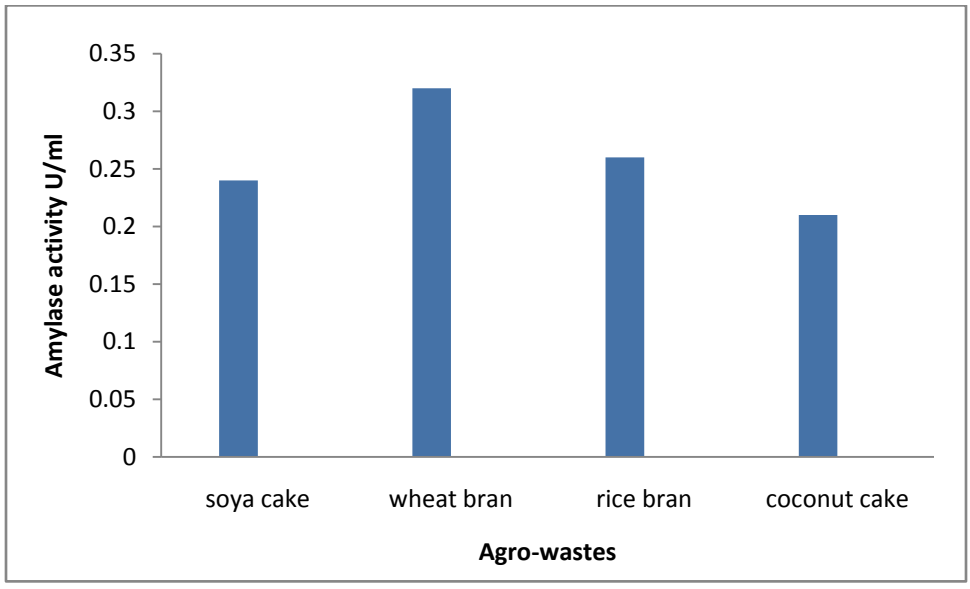

Fig.4:Effect of Agrowaste on Amylase production

IV CONCLUSION

The fungal strain *Aspergillusniger* was isolated and screened for Amylase production by clear zone formation by starch hydrolysis and selected for further studies .

The result suggested that fungal isolate *Aspergillusniger* is potential strain that can easily degrade starch .The effect of various processes parameters on Amylase activity was found to be influenced by incubation period , temperature pH and much more parameters . Maximum Amylase production during optimization process was achieved was at pH 8.0 , temperature 40˚ C and incubation period 6^{th} days . The present investigation showed that agro waste would be useful for exploitation and screening of amylolytic potential of fungal isolated .

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