I J R B A T, Sp. Issue (18) June 2021: 174-178
A Double-Blind Peer Reviewed & Refereed Journal
e-ISSN 2347 – 517X
Original Article
INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
 www.ijrbat.in

PRODUCTION AND CHARACTERIZATION OF CELLULASE PRODUCED BY ASPERGILLUS NIGER CULTURED ON WHEAT

Meshram R.L.[1] and Motwani D.R[1]
[1]. D R B Sindhu Mahavidyalaya, Department of Biochemistry, Panchpaoli, Nagpur.440017, Maharashtra, India.
*Correspondence: daminimotwani7@gmail.com

ABSTRACT:
Cellulases are enzymes that hydrolyse cellulose and related cellu-oligosaccharides derivatives. Wheat bran is the agricultural waste with the high cellulose content. The research was carried out to utilize wheat bran as media/substrate to produce cellulase enzyme by means of the fungi fermentation process. The crude cellulase enzyme was produced by *Aspergillus niger* through submerged fermentation process using wheat bran as carbon source. The optima fermentation conditions were determined by varying different parameters. In the research showed the cellulase enzyme activity of *Aspergillus niger* cultured on wheat bran media have optimum incubation period 6 days at pH 4.5 and temperature 40°C. Thepresent study has shown that wheat bran can be used as a carbon source by *Aspergillus. niger* for the production of cellulase.

Key words: - Cellulases, Wheat bran, Cellulose, *Aspergillus niger* and Submerged fermentation.

INTRODUCTION:

Agricultural waste is a big problem and its handling is very difficult as it contains too much cellulose which is difficult to degrade (Sonia N M O dan Kusnadi J 2015). Cellulose is a major component of cell wall of plants and it contribute approximately 35-50% of a plant dry weight (Saha B C 2004). Cellulose generate environmental pollution, and hence it is necessary to degrade cellulose polymer to be the simple monomers as glucose

(Septiani A and Wijanarkadan Rukmi M G I 2017). Cellulase (EC 3.2.1.4) hydrolyzes cellulose to glucose.

Fermentation technology is one of strategy that could be carry out in the effort to increase economic value of agricultural waste such as rice bran and wheat bran (Nema N, Alamir L and Mohammad M 2015). Wheat bran contain cellulose compound that can be used to produce cellulase enzyme that have a high economically value. Comercially enzyme production usually use fungi or bacteria (NarasimhaG, et al,2006). Cellulase enzymes have a variety of application in industry such astextile and laundry, food, animal feed, research and development, beer and wine, pulp and paper, agriculture, biofuel, pharmaceutical, waste management, and recombinant DNA technology (Behera B C,et al,2017).

The research is aimed to characterize a cellulase enzyme of fungal strain using wheat bran as substrate.

MATERIALS AND METHODS :

Isolation of fungal isolate

In view of getting efficient cellulose degrading fungal strains, soils rich in cellulose waste and fruit waste samples were scrutinized including fruit processing area,from different locations of Nagpur City. A suspension of soil sample and sterile distilled water was prepared , and plated on potato dextrose agar by (Mukunda et al,2012) . A broad spectrum antibiotic chloramphenicol , was use to inhibit bacterial growth . The plates were incubated at 28˚C for 2- 3 days .

Identification of fungal isolate

The isolated fungal strain was identified as *Aspergillus niger* based its morphological and microscopic characteristics and these values

matched withvalues in standard reference book compendium of soil fungi.

Screening for cellulase producers

The isolate was screened for cellulase activity. This was done by inoculating the organisms on the cellulase screening agar medium (CSAM)plates and incubated at 37°C for 7 days. Then the plates were flooded with congo red solution and incubated for 15min at room temperature. A clear zone of diameter above 1.386 around the growth of microorganisms indicated cellulase activity(Ugoh and Ljigbade,2013).

Production of cellulase of *Aspergillus niger*

Fermentation was carried out using *Aspergillus niger* for cellulase 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, cellulose-2g, Distilled water-100ml at pH5.0. 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 cellulase enzyme

The fermentated medium was centrifuged and cell free supernatend was used for estimation of cellulase activity .

Cellulase assay

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

RESULT AND DISCUSSION :

In the preliminary screening of the *Aspergillus niger* for the production of cellulase rapidly utilized cellulose with a halo diameter, this is an indication of cellulose production by the fungus. Several microorganisms have been reported to being endowed with vast potentials to produce arrays of enzymes (Jayani *et al.*, 2005; Varalakshmi*et al.*, 2007). The result of this study also revealed that all the commercial carbon sources supported the good growth of As*pergillus species* as well as the production of the enzyme cellulase by fermentation techniques (Motwani et al,2014). This is an indication that the fungus had the ability to utilize the various commercial carbon sources as substrates for growth. Moore–Landecker (1996) had earlier stated that fungi being heterotrophs obtain their required nutrients from the organic matter in the environment and thus are able to utilize a wide range of carbon substrates as source of energy. This ability has been attributed to the competitive saprophytic ability of fungi expressed by fast mycelia growth, spores production, presence of efficient and extensive systems of powerful enzymes (Silva *et al.*, 2005; Khalid *et al.*, 2006).

Optimization of culture conditions

Effect of incubation period on Cellulase production

Optimization of incubation period is an essential parameter for maximum growth of *Aspergillus niger* and hence greatly affects cellulose production.The results as shown in the **Figure:1** revealed that incubation period 6 th day was found to be best for cellulose activity . Cellulase 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* .

CONCLUSION:

Caffeine has been the topic of widespread investigation for its extensive past of usage plus prominent drinking global together in natural diets in addition to drugs. The mutual psychological as well as physiological effects of caffeine consumption be determined by mostly on the specific genotype and on the arrangement

plus the mark of acquaintance to the ingredient. The consumption of caffeine as an ergogenic encouragement must not be constrained through disclosure to hot atmospheres owing to worries of amplified liquid damage and amounts of heat storing. Caffeine is an operative approach to stand both cognitive as well as physical deprivation accompanying with sleep deficiency. It could be established that caffeine is a prospective regular, antimicrobial mediator in contradiction of different microorganisms, and so, could be recycled in nutrients as a natural protective, to regulate their development. This study shows various amount of caffeine present in different tea leaves available and used by the people of two different states of India.

Effect of Temperature on cellulase production

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

Similarly Spier et al,2006 reported that 45˚ C was optimum for cellulase activity by *Aspergillus species*.

Effect of pH on cellulase production

The effect of pH was studied by varying the pH of the medium from 3.0 to 6.0. pH affects the catalysis activity of cellulase enzyme Shah et al,2014. **Figure :3** shows that maximum cellulase activity was observed at pH 4.5 . pH changes the metabolic activity of cellulose producing strain .

CONCLUSION:

The fungal strain *Aspergillus niger* was isolated and screened for cellulase production by clear zone formation by cellulose hydrolysis and selected for further studies .

The result suggested that fungal isolate *Aspergillus niger* is potential strain that can easily degrade cellulose .The effect of various processes parameters on cellulose activity was found to be influenced by incubation period , temperature pH and much more parameters . Maximum cellulase production during optimization process was achieved was at pH 4.5 , temperature 40˚ C and incubation period 6th days . The present investigation showed that wheat bran would be useful for exploitation and screening of cellulolytic potential of fungal isolated.

REFERENCES:

Jayani, R. S., Saxena, S. and Gupta, R(2005), " Microbial pectinolytic enzymes: A review". Process Biochemistry. 40, 2931-2944.

Khalid, M., Yang, W., Kishwar, N., Rajput, Z. I. and Arijo, A. G(2006) " Study of cellulolytic soil fungi and two nova species and new medium". Journal of Zhejiang University SCIENCE B; 7(6), 459–466.

Miller GL(1959) " Use of dinitrosalicylic acid reagent for determination of reducing sugars". Anal Chem;31:426-8.

Moore-Landecker, E(1996) "Fundamentals of Fungi". 4th edn. Prentice Hall press, New Jersey, USA; pp. 279- 310.

Motwani,D.R,Meshram,V.G,Jambhulkar,V.SandMohadikar,M.R(2014),".Optimization of process parameters for utilization of orange peel for pectinase production by SSF using Aspergillus species".Astiatic journal of biotech.resources;SPL11,29 JAN,pp 56-60.

Nema N, Alamir L and Mohammad M(2015) Production of cellulase from *Bacillus cereus* by submerged fermentation using corn husks as substrates. *Int. Food Res. J.* 22(5)1831-1836

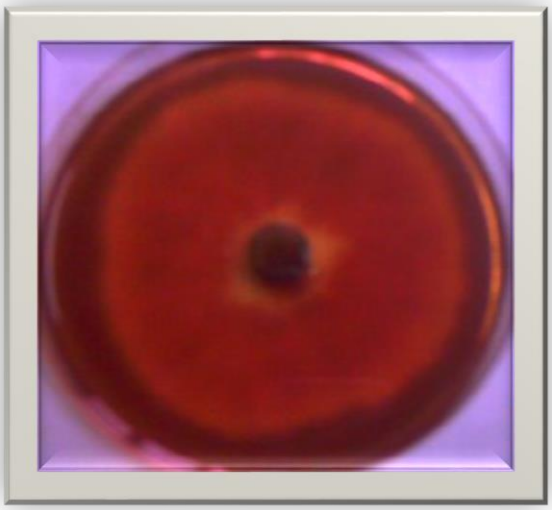
Saha B C (2004) *Lignocellulose Biodegradation and Application in Biotechnology*. US Government Work. Am. Chem. Soc. pp2-14

Septiani A, WijanarkadanRukmi M G I (2017) ProduksiEnzimSelulase Dari Bakteri*Serratiamarcescens*KE-B6 DenganPenambahanSumberKarbon, Nitrogen danKalsiumPada Medium Produksi*Bioma*19(2) 159-163 (inIndonesian)

Silva, R., Lago, E. S., Merheb, C. W., Macchione, M. M., Park, Y. K. and Gomes, E(2005) "Production of xylanase and cmcase on solid substrate fermentation in different residue by Thermoascus aurantiacus Miehe". Brazilian Journal of Microbiology; 36, 235-241.

Sonia N M O danKusnadi J (2015) Isolasi Danz KarakterisasiParsialEnzimSelulase Dari IsolatBakteri OS-16 Asal Padang PasirTengger-Bromo*J. Pangandan Agro.* 3(4) 11-19 (in Indonesian)

Varalakshmi, K. N., Anupama, J., Alva, S., Savia, J., Chiu, Y. Y., Vyshali, P., Shruti, M, Vieira, J. D. G., Barbosa, E. C. and Carrim, I. J. A(2006) "Enzymatic activity of endophytic bacterial isolates of Jacaranda decurrens cham. (Carobinha-do-campo) ". Brazillian Archives of Biology and Technology; 49(3), 353-359.



Clear zone formation on plate by the growth of *Aspergillus niger* showing evidence of cellulose degradation

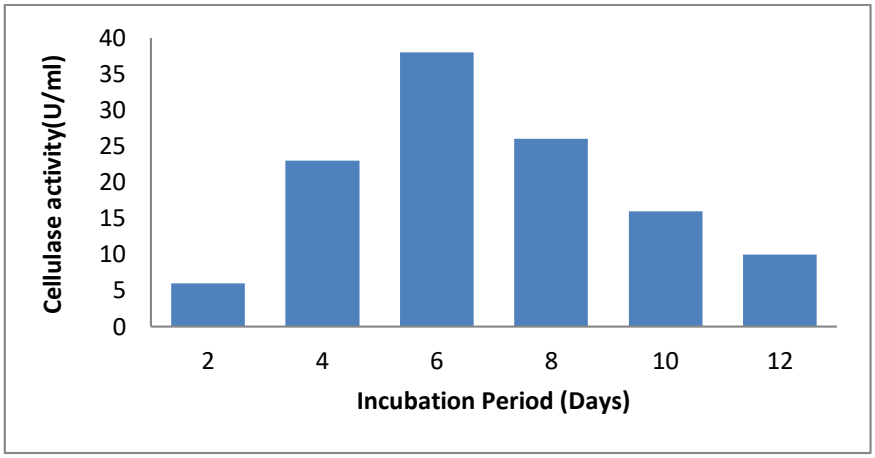


Fig.1: Effect of incubation period on Cellulase production

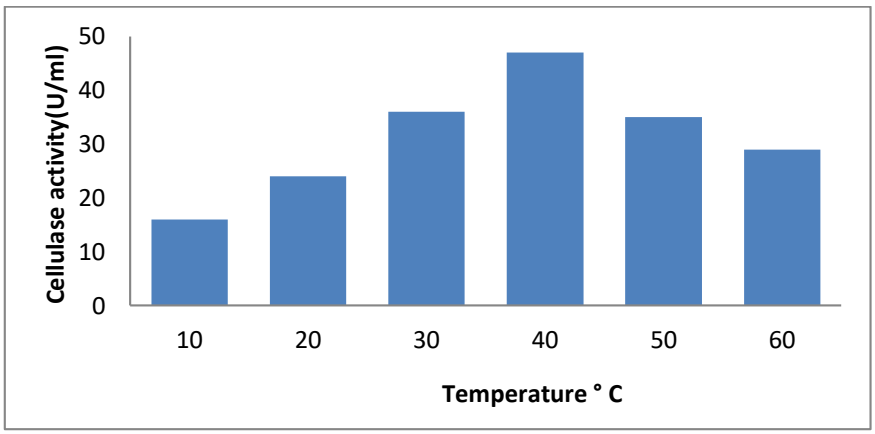


Fig.2: Effect of temperature on Cellulase production

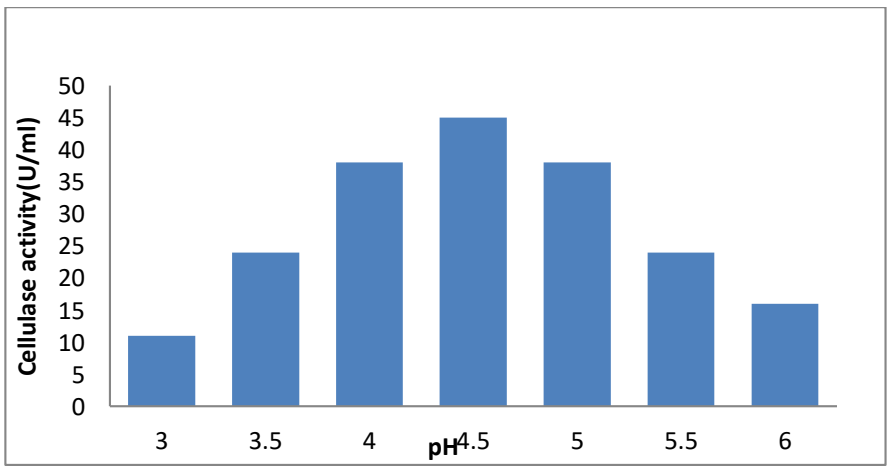


Fig.3: Effect of pH on Cellulase production