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Utilization of Agro Industrial Waste for Production of Protease by *Fusariumoxysporum* in Solid State Fermentation

KEYWORDS: Agoindustrial waste, *Fusariumoxysporum*, protease

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ABSTRACT *Agro industrial wastes constitute valuable source for microbial cultivation and enzyme production. Five agro-industrial wastes such as dal mill waste, oil mill waste, molasses, fruit waste and vegetable garbage were employed as substrates for the production of protease by Fusariumoxysporum. Protease enzyme was estimated after every 24 hrs of incubation which indicates the maximum protease production after 7 days of incubation in all the five agro industrial wastes. However dal mill wastes, oil mill waste, molasses, and vegetable garbage were favored most and showed production of almost similar amount of enzyme than in the fruit waste.*

Introduction

Recent developments in industrial biotechnology have resulted in the exploitation of new and undiscovered microorganisms and the devising of improved methods for enzyme production, which have led to increased yields of the enzyme, thus making a viable industrial process feasible (Sumantha et al.,2006). Environmental pollution by agro-industrial byproducts in developing countries can be alleviated by recycling into soils. However, little is known about their chemical composition and impact on soil fertility (Wakene Negassa et al., 2011). Annually around 998 million tones of agricultural waste are being produced globally. Only 15% is used as animal feed, particularly as supplement, plant fertilizer or soil conditioner while rest are dumped in landfills, which lead adverse environmental impact including global warming (Cauto and Sanroman,2005). In order to minimize the waste, one has to convert the waste to value- added products. Agro industrial waste contains fat, crude fiber, carbohydrates and protein and can be converted to useful agricultural and industrial products by microorganisms with the supplementation of nitrogen sources (Yang, 2002).Thus providing an alternative avenue and value addition to these otherwise under or non-utilized residues. They are considered the best substrates for the enzyme production in solid state fermentation (Ellaiah et al., 2002).

Materials & Method

Five agro-industrial wastes such as Dal mill waste, Oil mill waste, Molasses, Fruit waste and Vegetable garbage were employed as substrates for the production of protease by Fusarium oxysporum. All the five agroindustrial waste were collected from local area.

Substrate Preparation and Solid State Fermentation.

All agro industrial waste utilized in this study were powdered to size about 2 mm in homogenizer and then sieved through 20-40 mesh screens to obtain a particle having diameter between 0.42 to 0.85 mm. Each of the these 20 gm substrate was supplemented with 0.83gm K_2HP0_4, 0.16 gm $MgSo_4$, 1.5% agar-agar and 10 ml distilled water is then mixed with 1 ml spore suspension of 7 day old culture of Fusarium oxysporum and then poured in sterilized Petri plate for solid state fermentation. Incubation was carried out initially at room temperature (25-30°c) for 7 days.

Enzyme Estimation

During process of solid state fermentation over 7 day incubation period, after every 24 hrs of interval, 1 gm fermented substrate was harvested from Petri plate and transferred to test tube containing 5 ml phosphate buffer. The contents were homogenized and centrifuged at 2000 rpm for 30 min to remove all particulate matter. The culture filtrate after filtration through Whatmann filter paper was used for enzyme estimation. Protease activity was assayed as suggested by Keay and Wrildi, (1970) Protocol used and preparation of standard graph is mentioned as earlier.

Result and Discussion

Protease enzyme was estimated after every 24 hrs of incubation. Results are depicted in Table 1 and Fig.1 which indicates the maximum protease production after 7 days of incubation in all the five agro industrial wastes. However dal mill wastes, oil mill waste, molasses, and vegetable garbage were favored most and showed production of almost similar amount of enzyme than in the fruit waste. Megalla et al., (1990), achieved production of protease with Thermoas thermophilum after six days of incubation. In the study carried out by Lazim et al., (2009), protease activity was detected on 5th day of incubation. It was reported that maximal protease activity by Streptomyces sp. 594 was attained after the fourth day, reaching the level of 15.50 Ug^{-1} by Azeredo et al., (2005) Whereas in the case of S. rimosus, maximal protease activity (15.80 Ug^{-1}) was attained on the ninth day of incubation by Yang et al., (1999). Similarly Maximum protease activity production by Aspergillus flavus was obtained between the fifth and seventh day of incubation using SSF at 30°C by Malathi and Chakraborty, (1991).Maximal proteases was obtained after six days of incubation by A. terreus (Usama Ali, 2008). A. awamori showed a peak at 120 hrs of incubation (Negi and Banerjee, 2010). In a study by Divakar et al., (2006), Thermoactinomyces thalpophilus gave the maximum yield of protease at 72 hrs of incubation on a SSF process with wheat bran as the substrate.

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Table 1: Utilization of agro industrial waste for production of protease by Fusarium oxysporum in solid state fermentation.

Production of protease (µg/ml)at different Incubation period						
Type of waste	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.
dal Mill Waste	4.8	3.9	4.9	5.0	8.7	8.8
oil Mill Waste	2.6	2.1	2	3.4	6.5	8.1
fruit Waste	1.1	3.4	3.7	5.7	7.1	6.7
molasses	1	1.3	1.5	4.8	7.7	8.6
vegetable Garbage	3	3.5	6.7	8.2	8.7	8.8

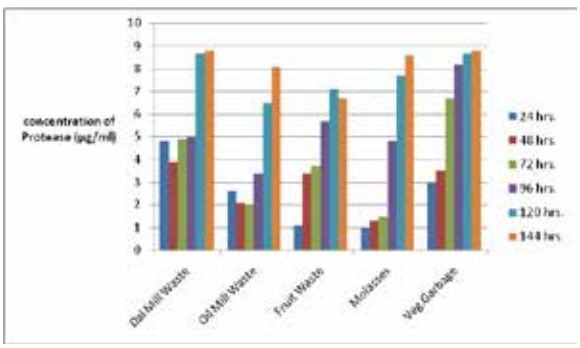

Fig 1: Utilization of agro industrial waste for production of protease by Fusarium oxysporum in solid state fermentation.

REFERENCE Azeredo C. De LAI, De Lima MB, Coelho RRR, Freire DMG, (2005), Thermophilic Protease Production by Streptomyces sp. 594 in Submerged And Solid-Sate Fermentation Using Feather Meal. J ApplMicrobiol 100:641–647. | Cauto, S. R., and Sanroman, M. A., (2005), Application of Solid State Fermentation to Ligninoly Enzyme Production. Biochemical Engineering Journal 22 (3):211-219 | Divakar G., Sunitha M., Vasu P. Uday Shankar P. and Ellaiah P., (2006), Optimization of | Process Parameters for Alkaline Protease Production Under Solid State Fermentation by Thermoactinomycesthalpophilus PEE 14.Indian Journal of Biotechnology Vol.5: pp 80-83. | | Ellaiah P, Adinarayana K, Pardasaradhi S, Srinivasulu B, (2002), Isolation of Alkaline Protease Producing Bacteria From Visakhapatnam soil. Ind J Microbiol 42:173–175. | | Keay,L. and Wrildi, B. S., (1970), Biotechnol. And Bioengg. 72:719. | | Lazim H., Houda M., Nedra S.,Insaf B., Ferid L.,(2009), Production and Optimization of Thermophilic Alkaline Protease in Solid State Fermentation by Streptomyces sp. CN902. J Ind. microbial Biotechnol. vol.36: pp 531-537. | | Malathis S, Chakraborty, (1991), Production of Alkaline Protease by a New Aspergillus flavus Isolate under Solid State Fermentation Condition for Use as a Depilation Agent. ApplMicrobiol 98: 491-497. | Megalla, S.E., A.M. MOharram, K.M. Abdel, (1990), Physiological Studies on Isolated from Poultry Feeds Tufs. Journal of basic Microbiology, 30(3):165-180. | Negi S. and R. Banerjee, (2010), Optimization of Culture Parameters to Enhance Production of Amylase and Protease FromAspergillus awamori in a Single Fermentation. African Journal of Biochemistry Research vol.4 (3), pp.73-80. | Sumantha A., Larroche C., Pandey A., (2006), Microbiology and Industrial Biotechnology of Food-grade Proteases: A perspective: Food technol. Vol.44 (2): pp 211-220. | | Usama F.Ali, (2008), Utilization of Whey Amended with some Agro-industrial by-products for the Improvement of Protease Production by Aspergillus terreus and its Compatibility with Commercial Detergents. Research Journal of Agriculture and Biological Sciences vol.4 (6): pp 886-891. | | WakeneNagassa, C. B. and Peter L., (2011), Soil Amendment with Agro industrial Byproducts: Molecular- Chemical Composition and Effects on Soil Biochemical Activities and Phosphorus Fractions. J. Plant Nutr. Soil Sci. vol.174: pp 113-120. | | Yang, S. S., (2002), Application of Solid State Fermentation in Agriculture and Industry. Scient. Agric., 50:156-167. | |