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STUDY THE EFFECT OF SUBSTRATE AND NON SUBSTRATE MEDIUM ON THE PECTINASE ACTIVITY OF FUNGI FROM VEGETABLES WASTE

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Abstract

Many plants cell wall contain pectin and it degrades by the microorganisms for their growth.Pectinase have been used in several conventional industrial processes over the years, such as textile, plant fiber processing and tea.In order to study the production of pectinase by ten dominating fungal species associated with vegetable waste were tested on substrate (pectin nitrate) and non substrate (glucose nitrate) medium. Pectinase activity was determined by viscometrical method.

The result revealed productions of pectinase were tested on substrate and non substrate broth medium. The fungi were subjected to different time intervals and pectinase enzymatic activity representing percent loss of viscosity. The highest pectinase activity was obtained by *Penicillium notatum*, *Trichoderma viride* and *Aspergillus niger* as compare to other fungi like *Aspergillus flavus, A. fumigatus, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Penicillium spp*. and *Rhizopus stolanifer* at different interval time 20, 40, 60 min.

Key word*:* Substrate, non substrate medium, Ostwald viscometer, fungi, pectinase activity, vegetable waste.

Introduction

The vegetables get spoiled by several biotic problems and an under threat due to fungi in all over at vegetables at market areas. Soil-borne plant fungi cause significant damage to almost all crops particularly to the vegetables (Usman *et al.,* 2013).In India, 20-30 % of the produce is spoiled in the markets (FAO, 2002; Deka *et al*., 2006). A huge amount of these materials to be decomposed by microorganism such as bacteria and fungi. Pectinase enzyme are produced by microorganisms including fungi, yeasts and bacteria, (Cao *et al.,* 1992; Blanco *et al.,* 1999; Huang and Mahoney,

1999). Pectinase, include a group of enzymes that are responsible for the degradation of pectin substances and have important applications in the food industry (Sandri, I.G. et.al, 2011)& (Sandri, I.G.et.al, 2013).

Now a day, pectinase enzyme is one of the most important enzymes in food processing industries mainly for extraction and clarification of fruit juices and wines (Oyewole et al., 2011). Pectinase have been used in several conventional industrial processes over the years, such as textile, plant fiber processing, tea and coffee industries, oil extraction, treatment of industrial waste water, containing pectinacious material, and paper manufacturing (Jayani et al, 2005).

Application of agro-industrial residues as carbon sources in enzyme production processes reduces the cost of production, and also helps in solving problems with their disposal (Murad and Azzaz, 2010).Such residues have yielded good results in the production of pectinase (Silva*et al.*, 2002). Pectinase constitute approximately 10% of the total enzyme production in the world market and 25% of global sale in the food industry, Naderi, S. et.al (2012).

In present investigation attempts were made to study the pectinase enzymes activity by dominating fungi from vegetable waste.

Material and Methods

Production of pectinase

Production of pectinase was made by growing the fungi in liquid medium containing pectin – 10gm, KNO_3 – 0.25%, $KH2PO_4$ – 0.1%, $MgSO_4.7H_2O$ – 0.05%, pH – 5.0. Out of which 25 ml of medium was poured in 100 ml Erlenmeyer conical flasks and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 1 ml standard spores suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at 25^0C with diurnal periodicity of light. On 7^{th} day, the flasks were harvested by filtering the contents through Whatman filter paper no.1. The filtrates were collected in the presterilized bottles and termed as crude enzyme.

Assay for pectinase

Pectinase activity was assayed by viscometric method (Papdiwal et. al, 1982) as viscosity loss % after 60 minutes. The Ostwald's viscometer was thoroughly cleaned with distilled water and dried before use. 6ml of pectin in 2ml of 0.2 M acetate buffer (pH 5.2) and 4ml of enzyme source were taken in viscometer and were thoroughly mixed and incubated at 25^0C temperature. The efflux time of the mixture at 0, 20, 40and 60 minutes was recorded with the help of stop watch.

The percent loss of viscosity was calculated by using the formula,

$$\text{Percent loss of viscosity} = \frac{T_0 - T_x}{T_0 - T_w} \times 100$$

Where, T_0 = Flow time in seconds at zero time

T_x = Flow time of the reaction mixture at time T

T_w = Flow time of distilled water

Biostatic analysis

All the results were statistically analyzed using analysis of variance (ANOVA) test and treatment means were compared using the least significant difference (C.D., p = 0.05) which allowed determination of significance between different applications (Mungikar, 1997) .

Results and Discussion

In present investigation production of Pectinase by ten dominating fungal species associated with Vegetable waste were tested on substrate (pectin nitrate) and non substrate (glucose nitrate) medium. On the contrary of this pectinase activity of *Aspergillus niger, A. flavus, A. fumigatus, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Penicillium notatum, P.spp, Rhizopus stolanifer and Trichoderma viride* was stimulated due to pectin.

Photoplate : Fungi cultured on liquid medium

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Table1: **Effect of non-substrate media on production of pectinase**

Fungi	**Non-substrate medium % (Glucose) viscosity loss after min**			**Standard Error (SE)**	**Critical Difference (CD) p= 0.05**
	20	40	60		
Aspergillus niger	41.93	44.83	46.12	**1.24**	**5.33**
Aspergillus flavus	03.37	04.77	05.76	**0.69**	**2.97**
Aspergillus fumigatus	03.93	07.12	08.59	**1.38**	**5.93**
Alternaria alternata	22.29	23.99	35.66	**4.20**	**18.06**
Curvularia lunata	20.14	22.30	25.41	**1.53**	**6.58**
Fusarium oxysporum	17.82	22.48	28.29	**3.03**	**13.03**
Penicillium notatum	03.94	07.17	09.35	**1.57**	**6.75**
Penicillium spp.	12.63	15.25	17.64	**1.45**	**6.24**
Rhizopus stolanifer	11.10	14.32	17.90	**1.96**	**8.43**
Trichoderma viride	02.21	04.42	05.65	**1.01**	**4.34**

Graph 1: Effect of non-substrate media on production of pectinase

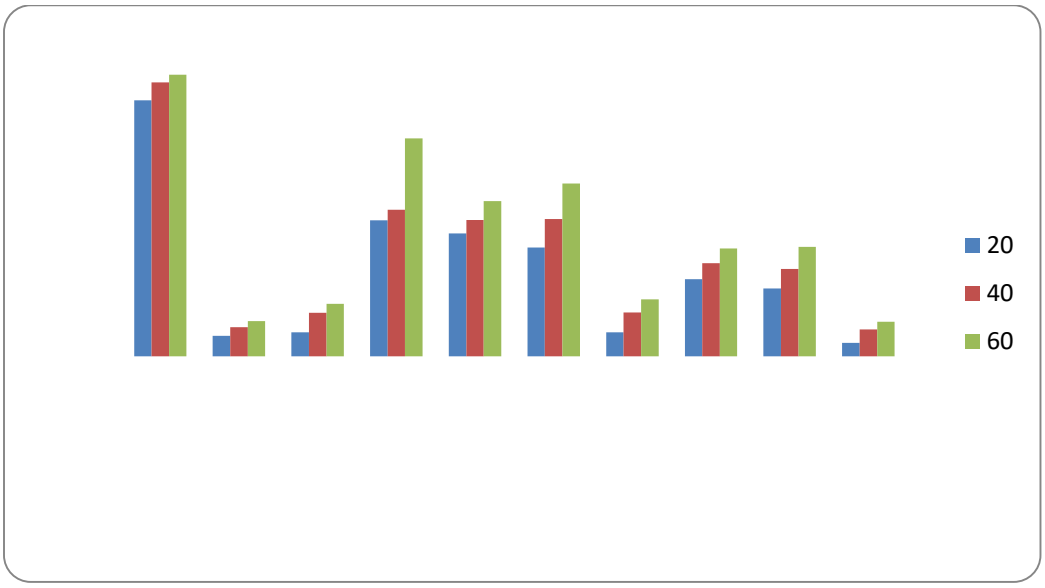
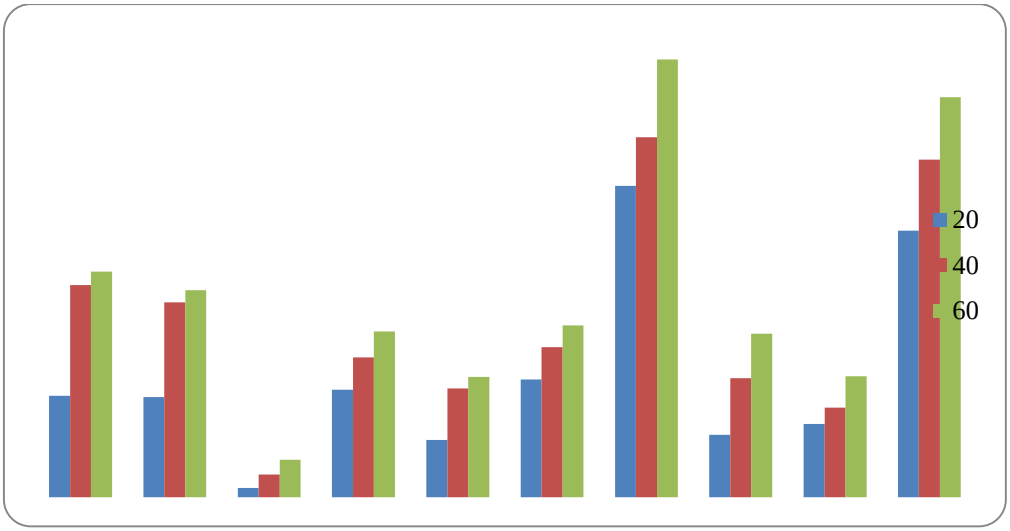


Table 2: **Effect of substrate media on production of pectinase**

Fungi	**Substrate medium % (Pectin) viscosity loss after min**			**Standard Error (SE)**	**Critical Difference (CD) p= 0.05**
	20	40	60		
Aspergillus niger	19.67	41.16	43.77	**7.64**	**32.85**
Aspergillus flavus	19.39	37.79	40.13	**6.56**	**28.21**
Aspergillus fumigatus	01.80	04.38	07.21	**1.56**	**6.71**
Alternaria alternata	20.85	27.13	32.16	**3.27**	**14.06**
Curvularia lunata	11.11	21.11	23.33	**3.76**	**16.17**
Fusarium oxysporum	22.80	29.07	33.33	**3.06**	**13.16**
Penicillium notatum	60.37	69.81	84.90	**7.14**	**30.70**
Penicillium spp.	12.05	23.07	31.73	**5.69**	**24.47**
Rhizopus stolanifer	14.22	17.36	23.43	**2.70**	**11.61**

Trichoderma viride	51.72	65.51	77.58	**7.47**	**32.12**

Graph 2: **Effect ofsubstrate media on production of pectinase**



The production of pectinase was determined by measuring percent loss of viscosity by the mixture of culture filtrate of substrate and non substrate broth media. The observed records were noted in the table .The percent loss of viscosity was obtained as a particular time intervals. It was cleared from the results table & graph no.1 & 2, summarized that loss of viscosity was directly related with the time. As time interval increased more viscosity loss was observed.

From the resultMaximum pectinase activity was detected in non- substrate broth medium from *Aspergillus niger* (46.12% at 60 min.) and minimum (43.77% at 60 min) was showed in substrate medium. Final reading of viscosity loss was increased at time interval. Maximum pectinase activity was detected from *Aspergillus flavus*(40.13 %) in substrate medium and resistant (05.76 % at 60 min) in non-substrate medium. *Aspergillus fumigatus* (08.59% at 60 min), also showed highest pectinase activity in non substrate medium and minimum (07.21% at 60 min)

in substrate medium.The extracellular pectinase activity by *Alternaria alternata* (35.66% at 60 min) in non substrate and (32.16% at 60 min) were exhibited at similar rate. However, *Curvularia lunata* (25.41% at 60 min) showed maximum pectinase activity in non substrate medium, than others (23.33% at 60 min). *Fusarium oxysporum* (33.33% at 60 min) was highest pectinase activity recorded in substrate medium and lowest (28.29% at 60 min) was showed in non substrate medium. The highest pectinase activity obtained from substrate medium by *Penicillium notatum* (84.90 % at 60 min) and lowest found in non- substrate medium. Pectinase activity by *Penicillium spp.*(31.73% at 60 min) also increased in substrate medium and decreased (17.64% at 60 min) in non substrate medium. Maximal pectinolytic activity shown by *Rhizopus stolanifer* (23.43% at 60 min)in substrate mediumand minimum in (17.90% at 60 min)nonsubstrate medium. The maximum Pectinase activity detected by *Trichoderma viride* (77.58% at 60 min) in non substrate medium and minimum was showed in substrate medium.

Pectinase activity representing percent loss of viscosity and as time intervals were increased viscosity loss was increased progressively. Similar results were reported by Rathod, 2011, (Bhale and J. N. Rajkonda 2012).

It can concluded from table and graph, that pectinolytic enzyme production was found to be maximum in both substrate and non substrate broth media for all tested fungi in general. *Trichoderma viride* and *Penicillium notatum* showed highest pectinase production on non substrate medium compared to the other fungi. Substrate medium also respond to high pectinase production by *Aspergillus niger* and *Alternaria alternata*. This could be highly beneficial for the production of pectinase from Vegetables waste by fungi.

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