Short communication

Microbial pectinase from tropical fruit wastes

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Abstract

Possibility of producing pectinase utilizing fruit wastes of cashew, banana, pineapple, and grape under controlled fermentation with *Aspergillus foetidus* was studied. Among the different media composition tried, medium containing 5 g fruit waste + 0.05 g urea + 0.25 g ammonium sulphate supported better growth of the microorganism. Enzyme production was maximum in the medium with grape waste. As extractant of the enzyme, distilled water was better than $CaCl_2$. The ideal temperature and duration of fermentation were 40°C and 8 days respectively.

Keywords: *Aspergillus foetidus*, Fruit waste utilization, Pectin esterase activity, Solid state fermentation.

Biowastes are highly perishable materials and their disposal often is a problem in processing industries. Extraction of enzymes from biowastes using the technology of fermentation, which gained importance recently (Nottingham University, 1997), is one of the many ways of exploiting them profitably (Anand and Mainy, 1997). Moreover, enzymes have enormous demand in textile, detergent, wool processing, beverage and food processing industries (Landbo and Meyer, 2001). Among the different enzymes, pectinase is important in the food processing industry and it is estimated that the pectinase market in the world for industrial processes is about 1000 million pounds per annum (Singh, 2000). Pectinase can be induced in the medium that contain pectin through fermentation using a suitable microorganism. Although microbial pectinase is widely used in food processing industries in other countries, it is still in its infancy in India mainly because of the high costs involved. If economically viable technologies for production are available, it will promote the food processing industries in this country. The present study was taken up to ascertain the possibility of utilizing fruit wastes of cashew apple (*Ancardium occidentale* L.), banana (*Musa* spp.), pineapple (*Ananas comosus* (L.) Merr.), and grape (*Vitis* spp.) for pectinase production through solid state fermentation technology and to standardize the conditions for production of this enzyme with respect to media composition, temperature, extractant, and duration of fermentation.

Fruit wastes of cashew apple, banana, pineapple and grape (100 kg each), which are good sources of pectin (Vilasachandran et al., 1982; Srivastava and Kumar, 1994; Madhav and Pushpalatha, 2002), were collected from the processing unit at Vellanikkara and dried in open air to bring down the moisture content (20 to 30%). The samples were further dried in the hot air oven at 60°C till the moisture level was 8 to 10%. The dried samples were pulverized and sieved through a fine mesh of 10 mm size. The media was prepared by mixing 5 g sample of each waste powder with 2 g agar (dissolved in 100 ml of distilled water), at two levels of $CO(NH_2)_2$ (0.05 and 0.075 g), and $(NH_4)_2SO_4$ (0.25 and 0.3 g), resulting in 16 treatment combinations (four media per waste material and four waste materials). The media

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were autoclaved at 121°C for 20 min. and allowed to cool to ambient temperature. *Aspergillus foetidus* culture obtained from IMTECH, Chandigarh was inoculated on the media at a spore suspension rate of $2x10^7$ g^{-1}. The microorganism is reported to have potential for producing pectinase through solid state fermentation of pectin containing media under favorable conditions (IMTECH, 2003). The growth of the microorganism was assessed daily based on the diameter of the colony developed. The medium which supported more growth of hyphae was selected as the best. The selected media were taken in trays of 10x7x4 cm size, autoclaved, cooled, and inoculated with a spore suspension of *Aspergillus foetidus* at the rate of $2x10^7$ g^{-1} of dry material. To determine the ideal temperature and duration of fermentation, the trays were kept at 25, 30, and 40°C for 5 and 8 days. The enzymes from the fermented wastes were extracted with $CaCl_2$ (2%) and distilled water after 5 and 8 days. In each case, extraction was done twice and the extracts were pooled and centrifuged at 1000 rpm in a cold centrifuge maintained at 6–8°C and the supernatants were used. The pectin esterase activity in the extract was determined as suggested by Talboys and Busch (1970). The data were analysed in CRD and subjected to DMRT analysis using MSTATC package.

As can be seen from Table 1, the media containing the four fruit wastes and different proportions of nutrients supported *Aspergillus foetidus* growth. The growth was generally high in the medium with 5 g waste + 0.05g $CO(NH_2)_2$ + 0.25g $(NH_4)_2SO_4$ at all stages of observation; hence it was selected for growing *Aspergillus foetidus*. However, for cashew apple waste, *A. foetidus* growth was better when supplemented with 0.075g $CO(NH_2)_2$ and 0.25g $(NH_4)_2SO_4$ (6 days after inoculation). The growth of microorganism on the media is an indication of its capacity to utilize pectin contained in the fruit wastes (Madhav and Pushpalatha, 2002) and to produce pectinase. Determination of pectin esterase activity in the medium taken at different temperature for varying periods have shown that enzyme production was consistently high at 40°C (Table 2). Among the waste materials compared, grape waste had the highest values

Table 1. Growth of *Aspergillus foetidus* on different fruit waste-based media.

Medium[1] (Fruit wastes, 5 g)	Nutrient supplements (g)		Colony diameter (mm)			
	$CO(NH_2)_2$	$(NH_4)_2SO_4$	3	4	5	6
			(days after inoculation)			
Grape	0.05	0.25	4.85^{ab}	6.3^{ab}	8.00^{a}	8.65^{a}
	0.075	0.25	4.55^{bc}	5.60^{b}	7.10^{ab}	8.50^{a}
	0.05	0.3	5.05^{a}	6.80^{a}	7.90^{a}	8.80^{a}
	0.075	0.3	4.30^{c}	5.45^{b}	6.30^{b}	7.20^{b}
Banana	0.05	0.25	4.80^{a}	5.90^{a}	6.75^{ab}	7.20^{b}
	0.075	0.25	4.90^{a}	5.90^{a}	6.40^{b}	7.40^{b}
	0.05	0.3	5.20^{a}	6.30^{a}	7.30^{a}	8.80^{a}
	0.075	0.3	4.55^{a}	5.30^{b}	6.30^{b}	7.30^{b}
Cashew apple	0.05	0.25	4.95^{a}	6.05^{a}	6.45^{b}	7.70^{b}
	0.075	0.25	5.15^{a}	6.15^{a}	7.30^{a}	8.80^{a}
	0.05	0.3	5.15^{a}	6.15^{a}	7.20^{a}	8.80^{a}
	0.075	0.3	4.60^{a}	5.70^{a}	6.60^{b}	7.75^{b}
Pineapple	0.05	0.25	4.35^{c}	5.30^{bc}	6.00^{b}	6.55^{b}
	0.075	0.25	4.75^{b}	5.55^{b}	6.05^{b}	6.85^{b}
	0.05	0.3	5.20^{a}	6.30^{a}	7.25^{a}	8.05^{a}
	0.075	0.3	4.30^{c}	5.20^{c}	5.85^{b}	6.45^{b}

Means with the same superscript do not differ significantly.

[1]Composition of media: 5 g fruit wastes + 2 g agar+ $CO(NH_2)_2$ + $(NH_4)_2SO_4$ at the quantities specified as above.

Table 2. Pectin esterase activity in different fruit wastes eight days after inoculation with *Aspergillus foetidus* at varying temperatures.

Medium	Pectin esterase activity ([U] mg^{-1})		
	25°C	30°C	40°C
Grape waste	0.23^{ab}	0.30^{a}	0.35^{a}
Banana waste	0.24^{a}	0.26^{b}	0.28^{b}
Cashew apple	0.24^{a}	0.24^{c}	0.29^{ab}
Pineapple waste	0.21^{b}	0.28^{ab}	0.30^{ab}

Means with the same superscript do not differ significantly.

followed by pineapple and cashew wastes. Pectinase being a substrate inducible enzyme (Singh, 2000), pectin in the fruit wastes induced pectinase activity in the presence of a suitable microorganism. Grape waste in particular is a good source of pectin (Srivastava and Kumar, 1994), and its utilization by the microorganisms under ideal conditions explains the enhanced production of pectinase. As an extractant, distilled water was

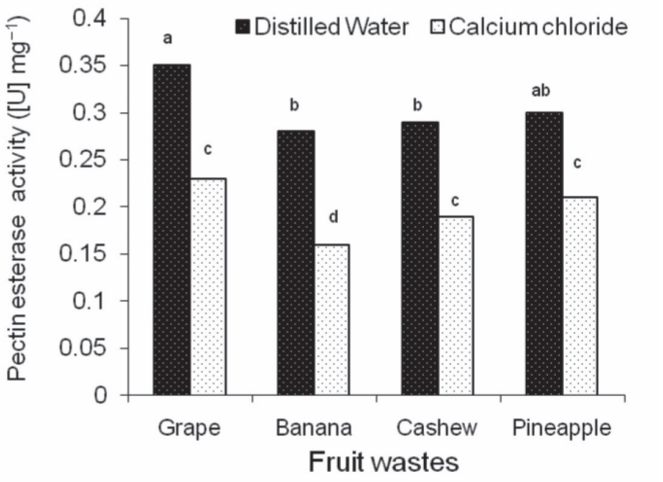

Figure 1. Pectin esterase activity in different fruit wastes inoculated with *Aspergillus foetidus* at 40°C of 8 days and extracted with distilled water and calcium chloride (means with the same superscript do not differ significantly).

superior to $CaCl_2$, regardless of the medium, temperature, and duration of fermentation. The highest pectin esterase activity was recorded (0.35 [U] mg^{-1}) in the medium containing grape waste at 40°C when extracted with distilled water at 8 days after inoculation (Fig. 1). Findings of the present study such as identification of appropriate sources of pectin (grape and pineapple fruit wastes), an ideal extractant (distilled water), and conditions for production of pectinase (fermentation for 8 days at 40°C) may favour pectin esterase production at lower costs.

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