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Exploration of Regional Agrowastes for the Production of Pectinase by *Aspergillus niger*

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Summary

The aim of this study was to evaluate locally available pectin rich agrowastes, *viz.* lemon peel, sorghum stem and sunflower head, as substrates for the production of pectinase by *Aspergillus niger* DMF 27 and *A. niger* DMF 45 in submerged fermentation (SmF) and solid-state fermentation (SSF) systems, respectively. The maximum amount of endo- (4.8 U/g) and exopectinases (17.2 U/g) was obtained from sunflower head followed by lemon peel (endopectinase 2.0 and exopectinase 10.2 U/g) in solid-state system. The increased level in the production of pectinases was noticed when the agrowastes were supplemented with additional carbon and nitrogen sources, and supplementation of sucrose was more effective than glucose in SSF. But, glucose yielded more pectinases in SmF. Among the nitrogen sources, ammonium sulphate raised the production level of pectinases from all the substrates in both SmF and SSF systems.

Key words: agrowastes, pectin, *A. niger*, submerged fermentation, solid-state fermentation, pectinase

Introduction

Pectinases are widely used in the food industries especially in the processing of fruits and vegetables (*1*) since they decrease the viscosity of juices and facilitate extraction, maceration, liquefaction, filtration and clarification processes. Pectinases are also used in the industrial processing of wine, coffee and tea fermentations (*2*). Production of enzymes from agrowastes could be important because they contain large amounts of cellulose, hemicellulose and pectin, which could serve as inducers for the production of cellulase, xylanase, and pectinases, respectively. Several agrowastes, mainly citrus peel (*3*), apple pomace (*4*) and coffee pulp (*5*) have been studied for the production of pectinases.

Submerged fermentation system has been extensively employed for the production of enzymes and to understand physiological aspects of the synthesis of the enzymes. However, SSF is mainly being advocated (*6,7*) to improve the production level of enzymes, because of several advantages over the SmF (*8,9*). Microorganisms are widely accepted as the best sources for the production of enzymes from agrowastes. Though bacteria are known to produce industrial enzymes, fungi are desired for the production of enzymes because their nature is generally regarded as safe (GRAS) (*10*). Recently, the production of pectinases from agrowastes (*11–13*) by fungi has been described as more attractive. Therefore, an attempt was made to examine the utility value of pectin rich regional agrowastes for the production of pectinases by *A. niger* in both SmF and SSF systems. The effect of the addition of carbon and nitrogen sources on the production of pectinases was also studied.

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Material and Methods

Substrates

Lemon peel, sorghum stem and sunflower head were obtained locally and were air dried, ground (particle size 300 μm) and autoclaved at 121 °C for 15 min. The pectin content of all these substrates was determined by gravimetric method (*14*).

Cultures

Two strains of *Aspergillus niger*, DMF 27 and DMF 45, were used for the production of pectinase in SmF and SSF, respectively (*15*). These were maintained on potato dextrose agar containing pectin and preserved at 4 °C. The spores were harvested from 96-hour-old cultures, grown at 30 °C in 0.01 % Tween 80 solution.

Fermentation

SmF was carried out in 250-mL Erlenmeyer flask by taking 100 mL of pectin medium containing (in g/L): $(NH_4)_2SO_4$ 0.1, $MgSO_4 \cdot 7H_2O$ 0.5, KH_2PO_4 0.5, and $FeSO_4 \cdot 7H_2O$ 0.0005 (*16*). pH of the medium was adjusted to 5.0. After sterilization at 121 °C for 15 min, flasks were cooled, inoculated ($1 \cdot 10^5$ spores/mL) and incubated on rotary shaker (200 rpm) at 30 °C. SSF was carried out in 250-mL flat bottom shallow glass container. The composition of the medium (by mass) was similar as above. The initial moisture content of the substrate was adjusted to 60 %. The pH and inoculum size of the medium were adjusted to 5.0 and $1 \cdot 10^7$ spores/g, respectively. The flasks were incubated at 30 °C for 72 h. The substrates were used independently in SmF and SSF to achieve 2 % pectin based on their natural amount of pectin.

Enzyme assay

The filtrates obtained from the flasks after incubation were stored at 4 °C for enzyme assay (*17*). Polygalacturanase activities were measured at 45 °C by viscometry (*18*) for endopectinase and by release of reducing sugars (*19*) for exopectinase. For endopectinase, a suitably diluted 1-mL sample was mixed with 18 mL of 2 % pectin in 0.1 M acetate buffer (pH=4.5), and the reduction in viscosity was monitored using Ostwald viscometer. Endopectinase unit (U) was defined as the amount of enzyme that reduces the viscosity of the solution by 50 % per minute under the conditions mentioned above. For exopectinase, a suitably diluted 0.3-mL sample was added to a solution containing 1 mL of 0.9 % of substrate and 0.7 mL of 0.1 M acetate buffer (pH=4.5). Reducing sugars were determined from the samples incubated at 45 °C for 30 min by dinitrosalicylic acid (DNS) method using galacturonic acid as reference. The quantity of the enzyme that liberates one micromole of galacturonic acid per minute under conditions mentioned above was defined as one exopectinase unit.

Results and Discussion

The experiments were conducted in triplicates and the results presented are the mean values. The pectin content of lemon peel, sorghum stem and sunflower head were 18.7, 12.8 and 21.4 %, respectively. Figs. 1 and 2 show the productions of pectinases from agrowastes by *A. niger* in SmF and SSF. The maximum amount of pectinase was produced from sunflower head, followed by lemon peel and then sorghum stem. Similar trend in the production of pectinase was observed in SSF system. The maximum level of pectinase production was achieved at 72 h in both systems, irrespective of the type of agrowastes employed. The exopectinase activity was higher when compared to endopectinase activity in both systems with all substrates.

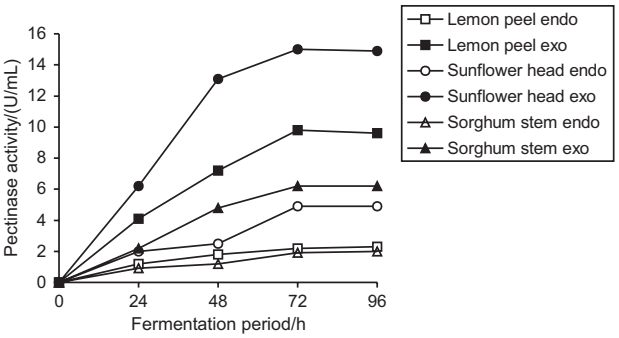

Fig. 1. Production of pectinases from agrowastes by *A. niger* in submerged system

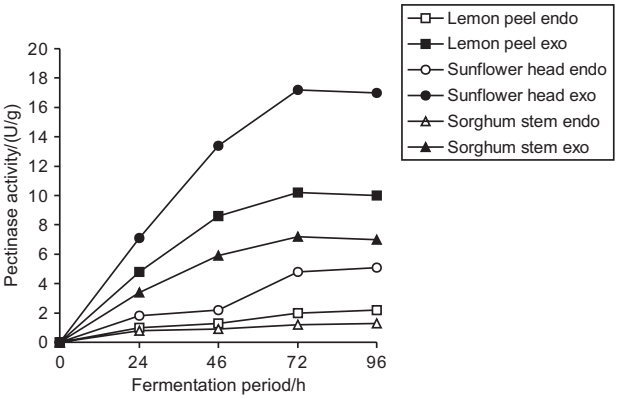

Fig. 2. Production of pectinases from agrowastes by *A. niger* in solid-state system

In general, any fermentation system is regulated by mainly physicochemical and nutritional factors. The nutritional parameters could be effectively monitored in the process for the maximum production of end product keeping physicochemical parameters as constant. Figs. 3–6 show the effects of the addition of glucose and sucrose as sources of carbon on the production of pectinases from all the substrates in SmF and SSF systems. Glucose induced the higher-level production of pectinase at 6 % concentration, whereas the requirement for sucrose was 8 % in both systems for all three substrates. Relatively higher amount of pectinase production was recorded in SmF with glucose when compared to sucrose, which yielded higher amount of pectinase in SSF system. The effects of glucose and sucrose on the production of pectinases in SmF and SSF have also been previously reported (*20,21*).

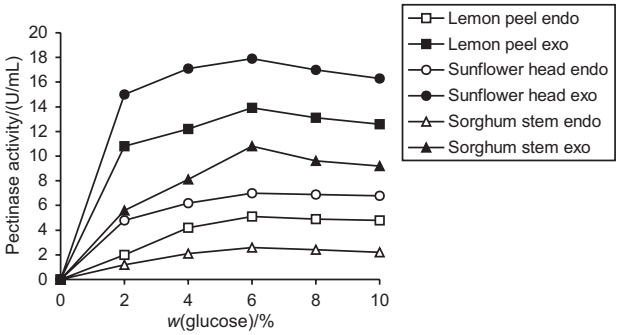

Fig. 3. Effect of glucose on the production of pectinases from agrowastes by *A. niger* in submerged system

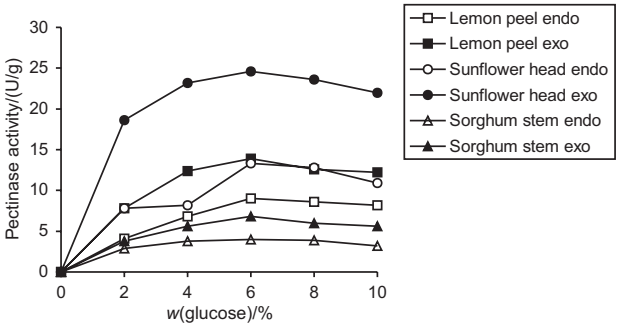

Fig. 4. Effect of glucose on the production of pectinases from agrowastes by *A. niger* in solid-state system

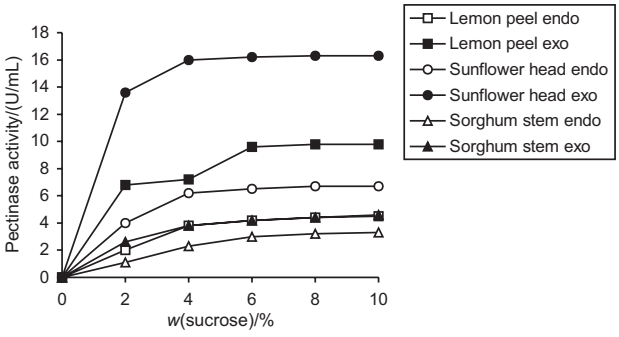

Fig. 5. Effect of sucrose on the production of pectinases from agrowastes by *A. niger* in submerged system

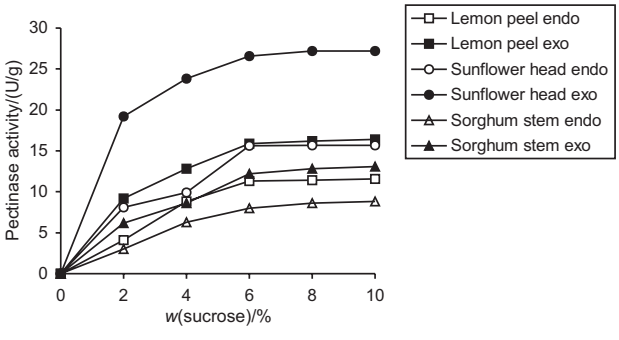

Fig. 6. Effect of sucrose on the production of pectinases from agrowastes by *A. niger* in solid-state system

Figs. 7–10 indicate the effects of ammonium phosphate and sulphate as nitrogen sources on the production level of pectinases in SmF and SSF systems. The maximum activity of pectinases was recorded at 0.3 % concentration in all three substrates in both systems. Ammonium sulphate and potassium phosphate have been reported to have no influence on the production of pectinases at lower concentrations (22). Of the both nitrogen sources in the present study, ammonium sulphate had greater influence on the production of pectinases from all the substrates in SmF and SSF systems.

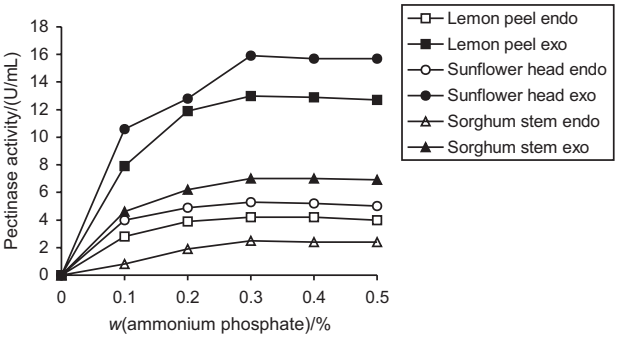

Fig. 7. Effect of ammonium phosphate on the production of pectinases from agrowastes by *A. niger* in submerged system

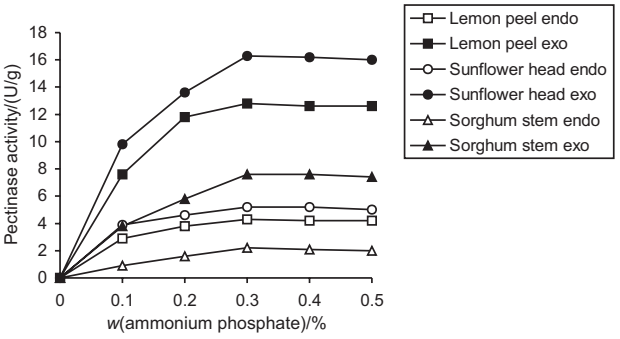

Fig. 8. Effect of ammonium phosphate on the production of pectinases from agrowastes by *A. niger* in solid-state system

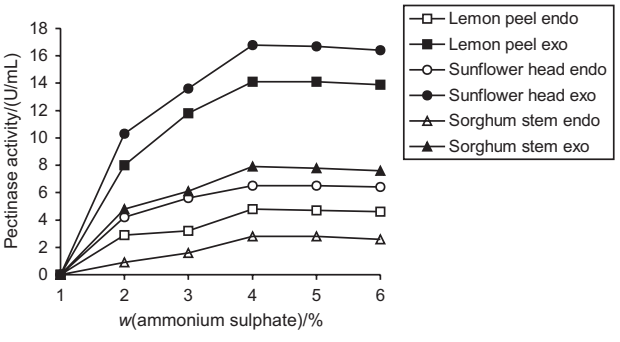

Fig. 9. Effect of ammonium sulphate on the production of pectinases from agrowastes by *A. niger* in submerged system

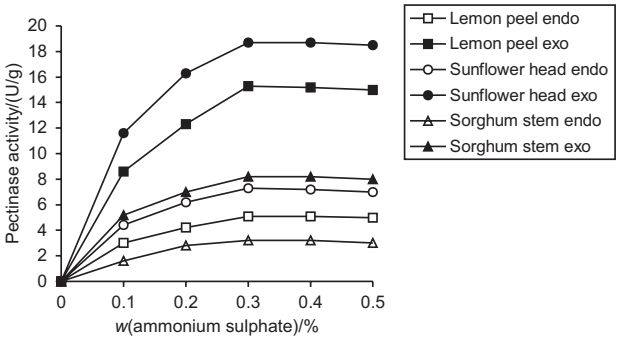

Fig. 10. Effect of ammonium sulphate on the production of pectinases from agrowastes by *A. niger* in solid-state system

Conclusions

The potential of pectinases solely as food enzymes is well known in the biotechnological industries because of their myriad applications. The production of these enzymes from agrowastes by fungi in solid-state system could not only be cost effective but it could also offer several process merits. Sunflower head and lemon peel largely available in the region could be effectively used as substrates for the production of pectinases. The results also showed that SSF could be advantageous over SmF for this purpose.

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