Malaysian Journal of Microbiology, Vol 9(4) 2013, pp.308-316
http://dx.doi.org/10.21161/mjm.52713

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Pomelo peels as alternative substrate for extracellular pectinase production by *Aspergillus niger* HFM-8

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Received 8 April 2013; Received in revised form 26 May 2013; Accepted 28 May 2013

ABSTRACT

Aims: The aim of this work was to develop an effective bioprocess to enhance the pectinase production by solid-state cultures of *Aspergillus niger* HFM-8.
Methodology and results: The pectinase production produced by *A. niger* HFM-8 was studied under solid state fermentation using Malaysian pomelo (*Citrus grandis*) peel as the substrate. This local agricultural waste is rich with lignocellulolytic material including pectin acts as the inducer of pectinase production. Under optimized conditions, 5 g of 0.75 mm pomelo peel size, moisture content of 60% (v/w) sterile distilled water pH 5.0, inoculums size of $1x10^4$ spores/mL, cultivation temperature of room temperature (30 ± 2 °C), no mixing incurred and with the addition of 1% (w/w) citrus pectin and 0.1% (w/w) urea has produced pectinase production of 306.89 U/g substrate and 0.78 mg glucosamine/g substrate of fungal growth on the 8th day of cultivation.
Conclusion, significance and impact of study: There was 48.82% increment in enzyme production after the improvement of parameters. It was found that pomelo peel is a suitable feedstock for pectinase production.

Keywords: Aspergillus niger, fungal pectinase, pomelo peels, solid state fermentation

INTRODUCTION

Pectin is a major structural carbohydrate of plant cell walls. Pectinases either hydrolyze glycosidic bonds of the galacturonic acid residues forming pectin chains or side groups. Major pectinases are polygalacturonase, pectin lyase, pectate lyase and pectin esterase (Ernesto *et al*., 2006). Pectinases are widely used in industrial processes that need solubilization of the cell wall of plants, wood, fruit or paper (Sarvamangala and Agasar, 2006). They are used in clarification and liquefaction of fruit juices (Reda *et al*., 2008), in textile industry to ret plant fibers such as cotton, jute, hemp and flax (Tzanov *et al*., 2001; Basu *et al*., 2009; Sharma and Mandal, 2012), in paper making industry to solve retention problems in mechanical pulps (Tohru *et al*., 2001; Reshmi *et al*., 2008) and in degumming of natural fibers (Kashyap *et al*., 2001) Pectinases are also uses in other process where elimination of pectin is essential such as in coffee, tea, and wine production (Gupta and Singh, 2004) and vegetable oil extraction (Demir *et al*., 2012).

Solid state fermentation holds tremendous potential for the production of enzyme (Aguilar and Huitron, 1990). Production of pectinases by solid state fermentation is an attractive proposition because of low water activity that discourages bacterial contamination. Agricultural wastes containing pectin can be considered as an alternative substrate for the production of pectinases. The substrate commonly used for pectinase production are of plant origin such as agricultural wastes which contain large amount of celluloses, hemicelluloses and pectin, which induced fungal production of the desired enzyme (Patil and Dayanand, 2006).

In Malaysia, pomelo (*Citrus grandis*) is widely grown in plantations and the rejected pomelo fruits and pomelo peels are discarded or composted. Sometimes, the rejected pomelo fruits are burnt, which leads to air quality problems. Furthermore, pomelo peels is reported to contain 16.9% soluble sugars, 3.75% fibers, 9.21% cellulose, 10.5% hemicelluloses, 0.84% lignin and about 42.5% pectin, 3.5% ashes, 1.95% fat and 6.5% protein (Rivas *et al*., 2008). Since pomelo peels contains an appreciable amount of pectin it should be a suitable feedstock for the production of pectinase by microorganisms under solid state fermentation system. Therefore, in this communication we are reporting the pectinase production by a local fungal isolate *A. niger* HFM-8 under solid state fermentation using pomelo peels as substrate. Filamentous fungi have been reported to have ability to synthesize high pectinase level under solid

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538

state fermentation and they are also preferred because of their ability to produce extracellular enzymes since high yield of enzymes can be easily extracted from culture medium.

MATERIALS AND METHODS

Microorganisms, culture maintenance and inoculum preparation

A. niger HFM-8, which was isolated from rotted oranges, was supplied by the Industrial Biotechnology Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. The fungal culture was maintained on potato dextrose agar (Merck, Germany) slant supplemented with 1.0% (w/v) citrus pectin (Sigma, Denmark) and incubated in incubator (Memmert, Germany) at 30 °C for five days aerobically (until sporulation) before storing them at 4 °C until further used. The stored cultures were sub-cultured transferred monthly to maintain viability.

The inoculum was prepared by adding 5.0 mL of distilled water to the agar slants and shaking vigorously. The spore suspension that obtained was adjusted to the desired concentration by using a haemocytometer slide chamber (Neubauer, Germany) and used as the inoculums.

Solid state fermentation and early profiling of pectinase production

Fresh pomelo peels used in this study were obtained from Changlun, Kedah, Malaysia have chopped into small pieces of uniform size and dried under sunlight until constant weight. They were then ground to powder form (specified particle size) prior of its use in solid state fermentation.

For cultivation of solid state fermentation, 5.0 g of (0.75 mm substrate size) pomelo peels was placed into a 250 mL Erlenmeyer flask and moistened with sterile distilled water (pH 7.0) at 80% (v/w). Then, $1x10^7$ spores/mL was added into the mixture and mixed gently with sterile spatula to spread the spores uniformly on the substrate. The inoculated flasks were cultivated for 14 days at room temperature (30 ± 2 °C) in a static condition without mixing.

Improvement of culture conditions and medium compositions for pectinase production and fungal growth by *A. niger* HFM-8

The strategy adopted for standardization of fermentation parameters was to evaluate the effect of an individual parameter and to incorporate it at optimum level for studying the effect of next parameter. Process parameters thus standardized included size of substrate (0.10, 0.50, 0.75, 1.00 and 2.00 mm), moisture content (50, 60, 70, 80, 90 and 100% v/w) different initial pH (2, 3, 4, 5, 6, 7 and 8), cultivation temperature (25, 30 ± 2, 35, 40 and 45 °C), inoculums sizes ($1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$ $1x10^7$ and $1x10^8$ spores/mL) and mixing frequency (no mixing, mixing once at every 12, 24, 48, and 72 h), various carbon (control without the addition of carbon source, glucose, fructose, lactose, sucrose, starch and pectin) (Fluka, Switzerland) and nitrogen (control without the addition of nitrogen source, ammonium sulphate, ammonium nitrate, ammonium chloride, peptone, yeast extract and urea) (Merck, Germany) sources. All experiments were performed in triplicates and the results were presented as means of the triplicate experiments.

Extraction of enzyme

The crude enzyme was extracted by mixing the harvested fermented materials with 50 mL of distilled water (Pandey *et al*., 1995). It was then mixed using orbital shaker at room temperature (30 ± 2 °C) and agitated at 150 rpm for 30 min. The medium was filtered using Whatman No. 1 filter paper and the cell free filtrate was used as the pectinase source of enzyme.

Enzyme assays

Pectinase assay was carried out using modified Dinitrosalicylic acid (DNS) method (Miller, 1959). The reaction mixture (1.0 mL) containing equal amounts of pectin (0.5%) prepared in citrate buffer (0.05 M, pH 4.5) and suitably diluted enzyme was incubated at 50 °C for 30 min in a water bath. After incubation, 3.0 mL DNS solution was added to stop the reaction and the tubes were kept in boiling water for another 10 min. On cooling, the developed colour was read at 575 nm using UV-visible spectrophotometer (Spectronic Unicam). The amount of released reducing sugar was quantified using D-galacturonic acid as a standard. The enzyme activity was defined as the amount of enzyme required to release one micromole equivalent of D-galacturonic acid per minute under assay conditions.

Determination of fungal biomass (glucosamine)

The fungal growth was determined using method previously described by Swift (Swif, 1973). The fermented sample was dried at 80 °C until constant weight and the glucosamine content was detected spectrophotometrically at 530 nm. The fungal growth was expressed as mg glucosamine per g of substrate. Glucosamine powder was used as a standard.

Statistical analysis

In order to evaluate and determine the significant of the findings and also to compare the differences among the findings, the statistical analysis was used. One way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) using PASW Statistics, version 18 were used to analyze the significant different of the mean of experimental data. A 5% confidence level or $\alpha = 0.05$ was used to test all experimental data. All enzyme

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activities and fungal growth were made in triplicates. The error bars showed the standard deviation of triplicates.

RESULTS AND DISCUSSION

Initial pectinase production and growth profiles before the improvement of parameters

The experiment was conducted in batch mode to determine pectinase production as a function of time. After about 24 h of cultivation the fungal mycelia appeared on the surface and at the end of 10 day, there was a rather thick woven layer of mycelia formed on the surface of substrate. Figure 1 shows the highest pectinase production was achieved at the 8th day of cultivation with the value of 206.22 U/g substrate and the fungal growth of 1.23 mg glucosamine/g substrate. It was observed that the production of enzyme increased over time from day 0 and peaked at day 8 of cultivation. The enzyme production decreased after achieving its maximal production. However, it was noted that the fungal growth achieved its maximum on day 10 of cultivation time. The results revealed that maximum biomass and enzyme production did not coincide.

This decline in enzyme production after achieving the maximum level could be a result of variation in pH of the medium during fermentation as a result of formation of organic acids (Shruti and Sudev, 2012). Also after a certain period of time the fungus may start hydrolyzing the enzymes for synthesis of biomass protein. Hours *et al*. (1988) reported that their fungal strain *Aspergillus foetidus* produced the maximal pectinase activity after 96 h (4 days) of cultivation on apple pomace. Generally, the cultivation time varies and it depends on many factors including difference of microorganisms and substrate used. In solid state fermentation, the substrate not only supplies the nutrients for cell growth but also provides anchorage to the microorganisms (Lonsane *et al*., 1985). Therefore, in the subsequent experiments, 8 days of cultivation time was used.

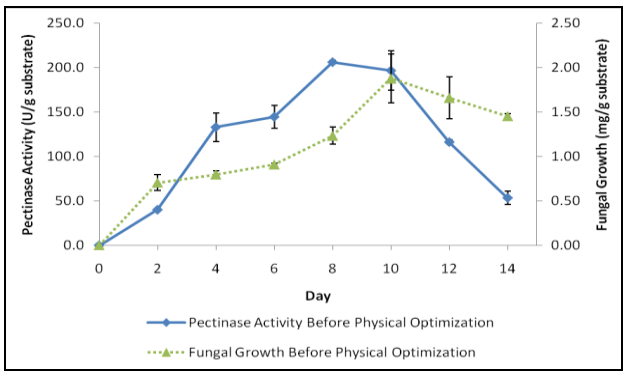

Figure 1: Time course profiles of pectinase production and the growth of *A. niger* HFM 8 before improvement of parameters. This experiment was carried out in triplicates. The error bars show the standard deviation values of the triplicates.

Effects of substrate size

The effect of substrate particle size on enzyme production and fungal growth were investigated. Figure 2 shows that substrate particle size of 0.75 mm produced the highest pectinase of 212.77 U/g substrate and 1.49 mg glucosamine/g substrate of fungal growth. Higher or lower substrate sizes level produced lower yield of enzyme productions. The fungal growth was also increased slowly with increased in substrate sizes and achieved its maximal growth at the substrate size of 0.75 mm, and decreased thereafter.

Optimal particle size is important in order to produce high yield of enzymes. The smaller particle sizes usually provides large surface area which help to mix the substrate with the microorganisms and other nutrients, However, small particle size may lead to clumping of substrate, resulting in reduced accessibility to nutrients and insufficient oxygen supply that finally lower yield of enzyme production. Similar observations on the effect of particle size on enzyme production in solid state cultures have been reported by Bhatti *et al*. (2007) and Mrudula *et al*. (2011). Membrillo *et al*. (2011) found that the geometrical ratio, shape and size of substrate (sugar cane bagasse fibers) strongly influence the packing density for solid state fermentation substrate with an impact in the production of extracellular enzymes, growth rate and composition changes in substrate.

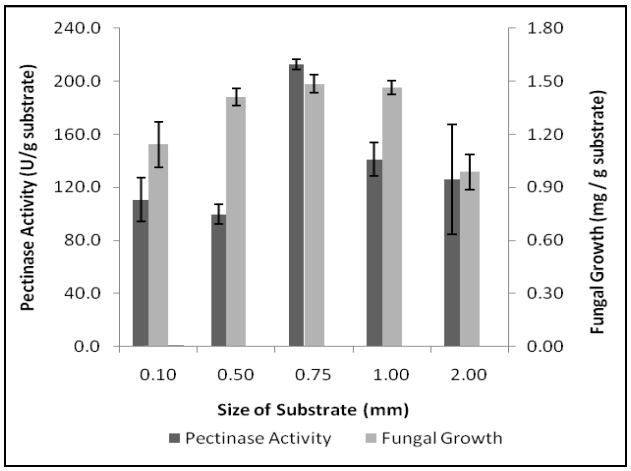

Figure 2: Effect of different substrate sizes on the production of pectinase and fungal growth by *A. niger* HFM 8. This experiment was carried out in triplicates. The error bars show the standard deviation values of the triplicates.

Effects of moisture content

The initial moisture content is an important factor that influences the growth and product yield in solid state fermentation. It is also depends on the type of microorganisms and substrate use in the cultivation. Furthermore, moisture is reported to cause swelling of the

ISSN (print): 1823-8262, ISSN (online): 2231-7538

substrates, thereby facilitating better utilization of the substrate by microorganisms.

It was observed that moisture content of 60% (v/w) yielded maximum pectinase activity of about 216.76 U/g substrate and fungal growth of 2.04 mg glucosamine/g substrate (Figure 3). Further increased in moisture content caused a significant decreased ($p \leq 0.05$) in enzyme yield.

Microbes generally grow near the outer surface of the substrate particle and at the same time evaporation of water takes place due to generation of heat during microbial growth. Hence the optimum humidity allows the entry of nutrients easily through the cell membrane, which favors maximum enzyme production. Any deviation from the optimum humidity results in the decrease in enzyme activity, which may be due to osmotic imbalance inside the cell causing cell lysis. Furthermore, higher moisture level leads to higher in humidity that would make the solid substrate clump together which results in the decrease of inter-particle space leading to decreased diffusion of nutrients (Babu and Satyanarayana, 1996; Venkateswarlu *et al*., 2000). In addition, higher moisture level also may cause reduction in enzyme yield due to reduction in porosity of the substrate thus interfering with oxygen transfer (Pérez-Guerra *et al*., 2003). In contrast, the low moisture content leads to the decreased solubility of nutrients present in the substrate, thus leads to poor microbial growth and thereby decreases enzyme yield. The optimum moisture content greatly depends on the water-binding characteristics of the substrate, temperature and selected microorganism (Syarifah *et al*., 2012).

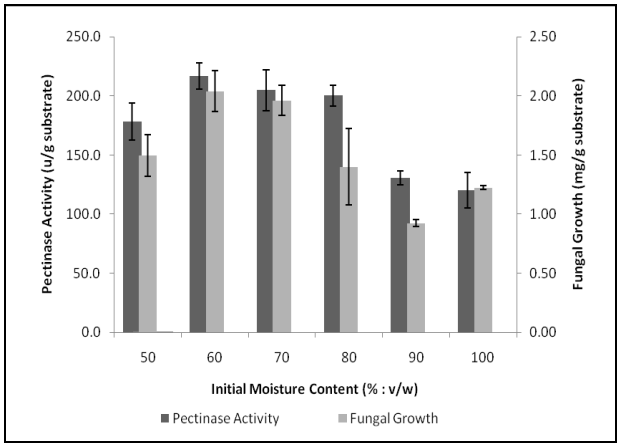

Figure 3: Effect of different ratios of moisture contents on the production of pectinase and fungal growth by *A. niger* HFM 8. This experiment was carried out in triplicates. The error bars show the standard deviation values of the triplicates.

Effect of initial medium pH of the moistening agent

In solid state fermentation, several of moistening agents have been used including distilled water. The pH of the medium which supplied by the moistening agent strongly affects many enzyme processes and transport of various components across the cell membranes (Abbasi *et al*., 2011). In this study distilled water was used as the moistening agent. Figure 4 shows that initial pH of 5 yielded maximum pectinase activity of about 270.91 U/g substrate and fungal growth of 1.00 mg glucosamine/g substrate. Further increase in pH value of the distilled water caused a significant decreased ($p \leq 0.05$) in enzyme yield. The initial pH of the distilled water affects the physiology of the microorganisms and therefore it was desired to determine the optimum pH value for the enzyme production.

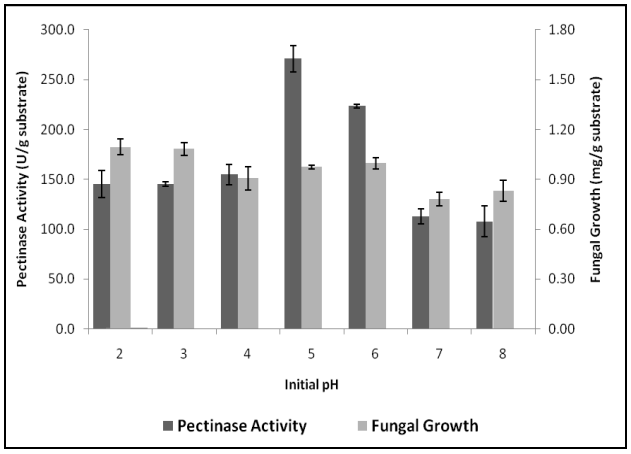

Figure 4: Effect of different ratios of moisture contents on the production of pectinase and fungal growth by *A. niger* HFM 8. This experiment was carried out in triplicates. The error bars show the standard deviation values of the triplicates.

The enzyme activity decreased with increase in pH of the substrate. Furthermore, optimal pH is important for microbial growth and their metabolic activities. Since the metabolic activities of the microorganisms are quite sensitive to changes in pH, pectinase production by *A. niger* HFM-8 was affected by varying pH values of the medium. Our findings are in line with the works of Ellaiah *et al*. (2002) and Muthuprakash and Abraham, (2011) who found pH 5 was the optimal culture condition for enzyme production by fungal strains including *Aspergillus* sp. under solid state fermentation. This acidic condition in the solid state fermentation was acceptable since the pomelo peels used as a substrate in this study was acidic by nature and it was favorable for the fungus to grow and produced enzyme.

Effects of cultivation temperature

The effect of temperature on enzyme production and fungal growth were examined. Figure 5 shows that the highest pectinase activity was observed at room temperature (30 ± 2 °C) with 225.87 U/g substrate and 1.13 mg glucosamine/g substrate. Higher or lower temperature than the optimum level produced less enzyme production and fungal growth. Malaysia is a

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

tropical country, therefore temperature at 30 °C is an ambient temperature, and by employing this temperature the fermentation cost can be saved up significantly. *A. niger* HFM-8 is a mesophilic fungus and was isolated from the rotten orange that discovered at the environment of 30 °C.

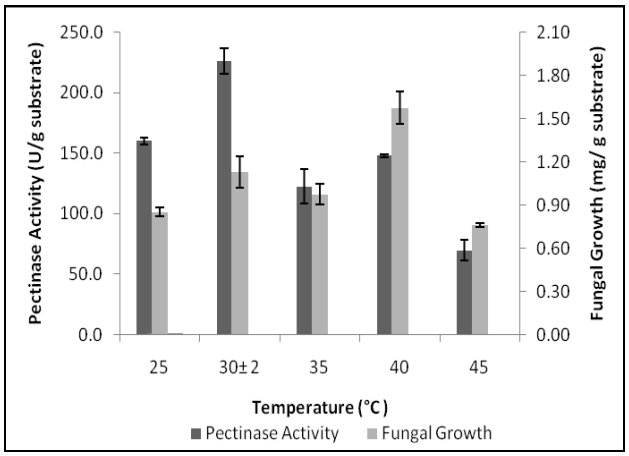

Figure 5: Effect of various cultivation temperatures on the production of pectinase and fungal growth by *A. niger* HFM 8. This experiment was carried out in triplicates. The error bars show the standard deviation values of the triplicates.

Temperature is a very important factor for microbial growth as well as microbial product formation and it varies from each of microorganism. In fact slight changes in growth temperature may affect pectinase production (Syarifah *et al*., 2012). That is why several experiments were carried out at different temperatures (25-50 °C) to see the effect of temperature on pectinase production and the results are shown in Figure 5, which indicated that maximal production of enzymes depend on cultivation temperature. However, the enzyme activities predominantly decreased due to inactivation of enzymes at temperatures higher than that of the optimum ones. Gummandi *et al*. (2007) reported an optimum temperature of 30 °C for pectin lyase production with *A. niger* NCIM548. Our strain of *A. niger* HFM-8 is a mesophilic fungus and therefore it can grow in the range of temperature between 25 to 40 °C. Lee *et al*. (2011) stated that the total cultivation temperature in substrate sometimes can be higher than the surroundings by as much as 20%.

Effects of inoculum size

Figure 6 shows that inoculum size of 1 x 10^4 spores/mL gave the highest pectinase production with 284.46 U/g substrate, and the fungal growth value of 0.81 mg glucosamine/g substrate. The findings also revealed that the higher or lower inoculum size than the optimum concentrations produced lower enzyme activity as well as fungal growth. The production of pectinase increased with an increase in inoculums sizes from 1x10^3 spores/mL and a further increase in inoculums size more than the optimized one did not favor significantly (p<0.05) produced higher enzyme production.

It is proven that fungi endure a contact between mycelia and substrate. Therefore, inoculum size is an important factor in a solid state fermentation processes since higher inoculum sizes, besides increasing spore concentration, also increase water content of the solid substrate, thereby inhibiting fungal growth and enzyme induction (Darah *et al*., 2012). On the other hand, lower inoculum sizes require longer time for fermenting the substrates in solid state fermentation cultures (Ramachandran *et al*., 2004).

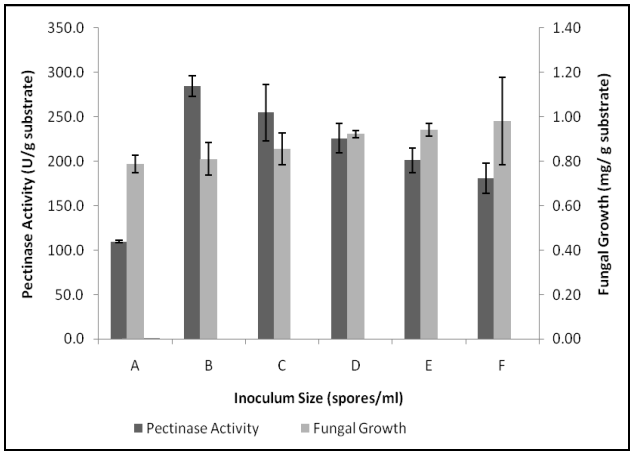

Figure 6: Effect of different inoculum size on the pectinase production and fungal growth by *A. niger* HFM 8. A= 1x10^3, B= 1x10^4, C= 1x10^5, D= 1x10^6, E= 1x10^7, F= 1x10^8. This experiment was carried out in triplicates. Error bars indicate the standard deviation values of the triplicates.

Effects of mixing frequency

The effect of various mixing frequencies on pectinase production and fungal growth were studied and the results are shown in Figure 7. The highest pectinase production with the value of 287.65 U/g substrate and the fungal growth of 0.96 mg glucosamine/g substrate was obtained when no mixing incurred. Mixing produced significantly lower enzyme production. In solid substrate the substrate size and shape may play important role in the effect of mixing of solids on the growth of filamentous fungi. In this experiment only 5.0 g of substrate was used and in this condition, mixing would result in particle injuries which finally affected growth of fungal mycelia and reduced the enzyme production. In previous works, we found that mixing was necessary when a larger amount of substrate was used where substrate thickness influenced the fungal growth and enzyme production (Syarifah *et al*., 2012; Darah *et al*., 2012).

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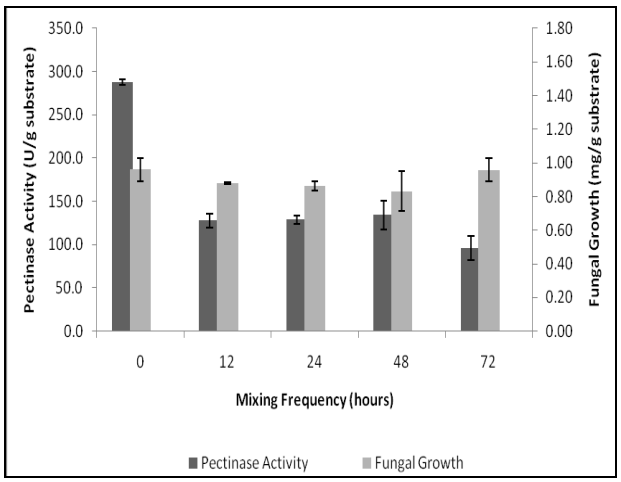

Figure 7: Effect of various mixing frequencies on the production of pectinase and fungal growth by *A. niger* HFM 8. This experiment was carried out in triplicates. Error bars indicate the standard deviation values of the triplicates.

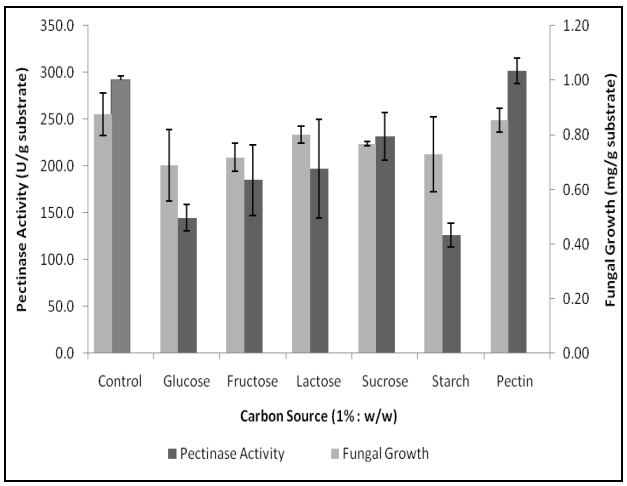

Figure 8: Effect of different carbon source on the production of pectinase and fungal growth by *A. niger* HFM 8. This experiment was carried out in triplicates. Error bars indicate the standard deviation values of the triplicates.

Effects of carbon source

The effect of using additional carbon source to pomelo peel was investigated and the results are shown in Figure 8. Among all the six types of carbon source tested, the citrus pectin induced the highest pectinase production which was 301.56 U/g substrate and fungal growth of 0.85 mg glucosamine/g substrate. Surprisingly, the control which consisted of pomelo peel only as a sole carbon source was the second highest in enzyme production with the value of 292.26 U/g substrate and the fungal growth was 0.88 mg glucosamine/g substrate. The third highest pectinase activity was produced in the presence of sucrose which was 231.40 U/g substrate and the fungal growth was 0.77 mg glucosamine/g substrate. The addition of lactose in the fermentation system only produced pectinase of 197.45 U/g substrate with fungal growth of 0.80 mg glucosamine/g substrate. The presence of fructose yielded about 184.91 U/g substrate and 0.72 mg glucosamine/g substrate. On the other hand, the addition of glucose and starch only produced about 144.68 and 126.24 U/g substrate of pectinase and 0.69 and 0.73 mg glucosamine/g substrate, respectively. Even though citrus pectin showed the highest value of fungal growth (0.85 mg glucosamine/g substrate), but in term of pectinase production, the value was no significant difference with the control (Duncan, $p<0.05$). Therefore, the addition of carbon source was neglected for the subsequent studies in consideration to save cost in the fermentation process. Pomelo peels alone is sufficient to be used as a substrate and also as carbon and inducer sources, since it is nutritionally rich with 16.9% soluble sugars, 3.75% fibers (9.21% cellulose, 10.5% hemicelluloses, 0.84% lignin and about 42.5% pectin, 3.5% ashes, 1.95% fat and 6.5% protein (Rivas *et al*., 2008).

Effects of nitrogen source

The source of nitrogen in the growth medium has very important role in fungal growth and enzyme production (Juwon and Emmanuel, 2012). The results of the present study showed that the best nitrogen source for the highest production of pectinase was urea (Figure 9) with 311.50 U/g substrate and 0.85 mg glucosamine/g substrate. The second highest of enzyme production was observed in the fermentation medium supplemented with ammonium nitrate with the value of 277.38 U/g substrate and the fungal growth of 1.01 mg glucosamine/g substrate. The addition of ammonium sulphate in the fermentation medium showed the third highest pectinase production with the value of 274.37 U/g substrate and the fungal growth of 0.86 mg glucosamine/g substrate. Lower pectinase production was observed in the control which was the absence of any nitrogen source with the value of 223.13 U/g substrate and the fungal growth of 0.89 mg glucosamine/g substrate. However, the presence of peptone and yeast extract in the fermentation medium only showed the enzyme production with 166.49 and 153.68 U/g substrate, respectively. Therefore, urea was used as a nitrogen source in the next experiment since it was significantly different with other type of nitrogen source according to Duncan, $p<0.05$.

Pectinases production under solid state fermentation was reported to enhance by the addition of nitrogen sources such as yeast extract, peptone and ammonium chloride but was inhibited by glycine, urea, ammonium nitarate (Fagerquist *et al*., 2005). However, in our study, the addition of 0.1% urea enhanced the enzyme production. Based on this result, urea was used since it reduced the cost of the fermentation process.

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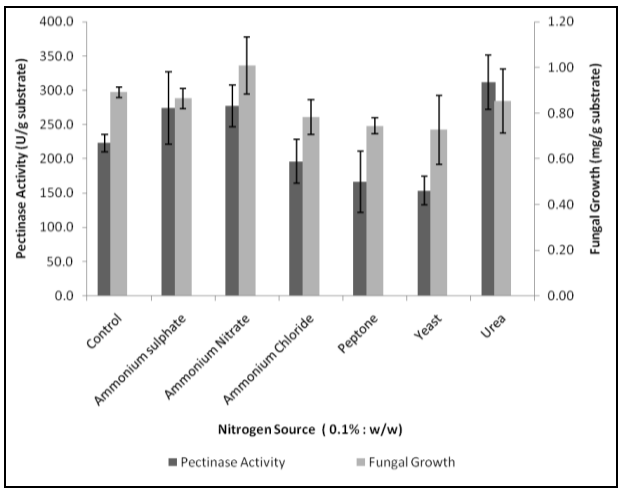

Figure 9: Effect of different nitrogen source on the production of pectinase and fungal growth by *A. niger* HFM 8. This experiment was carried out in triplicates. Error bars show the standard deviation values of the triplicates.

Effects of different concentrations of urea

The concentration of urea was varied from 0.0% to 0.5% (w/w). The highest pectinase production was observed after addition of 0.1% (w/w) urea with the value of 326.39 U/g substrate and the fungal growth was 0.77 mg glucosamine/g substrate (Figure 10). The addition of higher or lower urea concentration produced less enzyme production. Our study revealed that 0.1% (w/w) of urea enhance the pectinase production significantly (p<0.05).

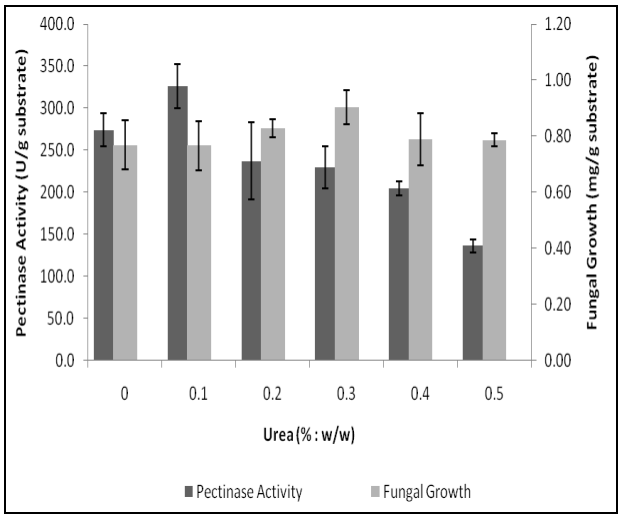

Figure 10: Effect of different concentrations of ammonium nitrate on the production of pectinase and fungal growth by *A. niger* HFM 8. This experiment was carried out in triplicates. Error bars show the standard deviation values of the triplicates.

Pectinase production and growth profiles after the improvement of physical and chemical parameters

All the optimized parameters obtained from the improvement of physical and chemical parameters were used and the time course profiles of the pectinase production and fungal growth were carried out for 14 days. Five grams of 0.75 mm of substrate particle size of pomelo peel was moisturized with 60% (v/w) of sterile distilled water, pH 5.0 in the addition of 0.1% (w/w) urea. The 1 x 10^4 spores/mL inoculum size was added into the substrate and mixed well. The inoculated flasks were incubated at room temperature (30 ± 2 °C) for 14 days in a static condition. Figure 11 shows the profile of pectinase production increased dramatically from day 2 to 6 while achieving the highest production on day 8 of cultivation (306.89 U/g substrate) with the fungal growth of 0.78 mg glucosamine/g substrate. However, the highest fungal growth was achieved on day 6 of cultivation time with the value of 0.93 mg glucosamine/g substrate. After achieving the highest pectinase production and fungal growth, the enzyme production decreased slowly until day 14 of cultivation. On day 10 of cultivation, pectinase production was 202.40 U/g substrate and the fungal growth was 0.75 mg glucosamine/g substrate. On the 12th day of cultivation time, the pectinase production decreased to 197.16 U/g substrate with the fungal growth of 0.73 mg glucosamine/g substrate. Subsequently, on day 14 of cultivation, only 101.15 U/g substrate of pectinase production was produced with the fungal growth of 0.69 mg glucosamine/g substrate.

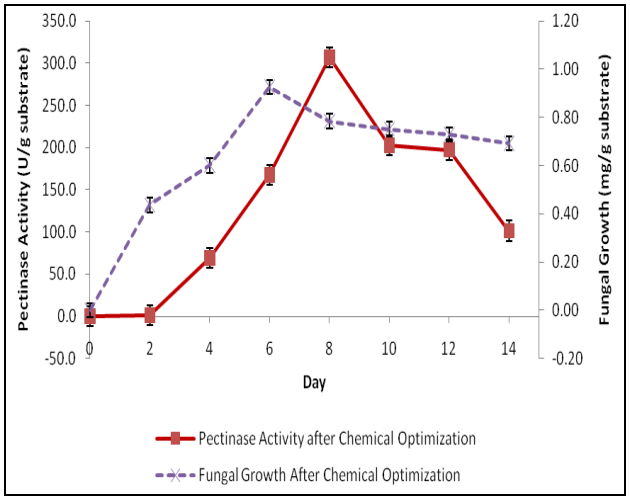

Figure 11: Time course profiles of pectinase production and fungal growth by *A. niger* HFM 8 under solid substrate fermentation using pomelo peels as substrate after physical and chemical improvements.

CONCLUSION

The cost-effective technologies are needed for the production of enzyme and solid state fermentation is a

ISSN (print): 1823-8262, ISSN (online): 2231-7538

suitable technology for economical production of pectinases using pomelo peels, one of the lignocellulosic residues as substrate. Major parameters affecting the fermentation process for enzyme production were studied and optimal levels were identified. It is concluded from the findings that the strategy to produce pectinases from pomelo peel was successful as it resulted in a considerable amount of enzyme produced by a newly isolated strain of *A. niger* HFM-8 under a laboratory conditions. To the best of our knowledge this is the first report of the use of pomelo peels as substrate for pectinase production in solid state fermentation. These results provided valuable information for the production of pectinases by *A. niger* strain using relatively inexpensive substrate.

ACKNOWLEDGEMENT

The authors would like to thank MOSTI and USM for providing research grants to conduct the project. Nor Hawani is thankful to Universiti Sains Malaysia for the RLKA of USM fellowship.

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538