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Assessment of Cultivation Parameters Influencing Polygalacturonase Production by *Aspergillus* sp. SO5 in Submerged Fermentation

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Abstract

Polygalacturonase represents an important enzyme of the pectinase consortium in the food and beverage industry. The present study aims to isolate, screen, and enhance physicochemical parameters for enzyme production under submerged fermentation. A total of 34 fungal strains were obtained from rotten fruits and 70.6 % of them are able to produce polygalacturonase by degrading pectin in pectin screening agar. The fungal isolate (SO5) that exhibited the greatest diameter of the hydrolysis zone (45.3 ± 0.04 mm) was selected for further study and later identified as *Aspergillus* sp. SO5, based on morphological characteristics using light microscopic observation. Several physicochemical parameters improved for pectinase production including incubation temperature, initial pH, agitation speed, inoculum density, and carbon and nitrogen sources. Under improved conditions, the polygalacturonase activity and fungal biomass increased up to 74.63 and 69.44 %, respectively. The finding also revealed that the improved physicochemical conditions enhance enzyme production and shorten the incubation period from 6 days to 5 days.

Keywords: Polygalacturonase, *Aspergillus* sp., Physiochemical parameters, Submerged fermentation

Introduction

Pectin is an acidic polysaccharide substance comprised of D-galacturonic acid that occurred in α-1, 4 chains, naturally esterified with natural sugars and methoxy groups occupying the side chains [1]. It is the main component in the cell wall and is present in the highest concentration in the middle lamella of fruits and vegetables since it plays an important role in cementing substances between adjacent cells [2]. Pectinase is a consortium of enzymes that catalyze the degradation of pectin. The most important pectinases are polygalacturonase, pectin-esterase, pectin lyase, and pectate lyase. These enzymes are mainly employed in the food and beverage industry to improve oil extraction, clarify fruit juices and wine, degum fibers, increase the firmness of some fruits, and remove peels from citrus fruits [3].

Polygalacturonase, most of the studied pectinase are enzymes that are involved in the degradation of pectin that catalyzes a-1, 4-glycosidic linkages hydrolysis in pectate or other galacturonans randomly in the middle (endo-polygalacturonase) or at the end (exo-polygalacturonase) of polymeric chains [4]. Polygalacturonase can be found naturally in plants and produced by several microorganisms with most commercial pectinase being produced from fungal sources [5]. *Aspergillus* sp. is the most studied and utilized fungal species for pectinase production at the industrial scale among the fungal strains. For instance, Karahalil [6] reported the utilization of *Aspergillus sojae* in polygalacturonase production with the enzyme activity of 34.55 ± 0.5 U/mL. Besides, other fungal strains that have been reported to produce pectinase include *A. niger* [7], *A. fumigatus* [8], *A. terreus* [9], *A. nomius* [10], and *A. awamori* [3].

Generally, polygalacturonase can be produced by submerged and solid-state fermentation. Both methods have advantages such as being cost-effective, higher yield of the enzyme, and ease of conduct. Darah [11] employed the solid-state fermentation system to produce pectinase from *Aspergillus niger* LFP-1 using pomelo peels as a sole carbon source. Meanwhile, other studies utilize orange peels to produce polygalacturonase by *Trichoderma harzianum* through submerged fermentation [12]. Therefore, the present study aimed to isolate the best fungal pectinase producer and investigate the optimum culture conditions and chemical compositions for polygalacturonase production by the best fungal strain in submerged fermentation.

Materials and methods

Isolation of fungal polygalacturonase producer

One g of whole rotten fruits (lemon, mango, orange, papaya, and banana) was introduced into a 50 mL Erlenmeyer flask containing 10 mL distilled water and the sample was shaken at room temperature for 10 min. One milliliter of the sample was diluted with 9.0 mL of sterile distilled water and the step was repeated to obtain a 10^4 final dilution. Aliquots (100 µL) of diluted samples were inoculated using the spread plate method onto sterilized potato dextrose agar (PDA) supplemented with streptomycin to prevent bacterial growth. The inoculated plates were then incubated at 30 °C for 7 days. After the incubation period, the plates were observed for fungal growth. To obtain pure fungal isolates, subculturing the plates with fungal growth was performed 3 times. The pure fungal cultures were maintained on a PDA slant supplemented with 1.0 % citrus pectin (w/v) at 30 °C for 3 days aerobically until sporulation, before storing them at 4 °C until further use. The subculturing was carried out once a month to ensure survivability and purity.

Fungal identification

A few mycelia of 3 days old pure culture were placed on the glass slide and a drop of cotton Lactophenol blue was added to it. A coverslip was placed over the sample and observed under a light microscope at 400X magnification. The fungal morphologies and features were compared with Klinch [13].

Preparation of spore suspension

The spore suspension was prepared by introducing 5.0 mL of sterile distilled water containing Tween 80 (0.1 %, v/v) to a sporulated culture and then dislodged by shaking vigorously and mixing using a sterile inoculation loop [14]. The spore suspension was adjusted to 1×10^7 spores/mL using a hemocytometer chamber (Neubauer Germany).

Culture medium and cultivation

A suspension (1 mL) with a spore density of 1×10^7 spores/mL was introduced into 250 mL Erlenmeyer flasks containing 100 mL of pectin broth (PB) medium and incubated at 30 °C for 10 days with an agitation speed of 150 rpm. Prior to that, the PB medium was prepared by introducing (w/v) 0.02 % $(NH_4)_2SO_4$, 0.007 % $MnSO_4$.7H2O, 0.007 %; and 1.0 % citrus pectin, into 1,000 mL distilled water, and pH was adjusted to 4.5 [14]. The cultures were withdrawn at every 24 h interval and were assayed for pectinase activity and fungal growth was determined.

Screening of polygalacturonase production

A small fragment of the fungal mycelia was point inoculated onto the pectin agar (PA) medium [consisted of (g/L): Pectin citrate 10.0, urea 0.05, ammonium sulfate 0.15, and agar 22.0 (pH 4.5)] and incubated at 30 °C for 3 - 4 days. A few drops of 1 % (w/v) cetrimide (cetyl trimethyl ammonium bromide) solution were flooded onto the agar after the incubation period to detect the presence of a hydrolysis zone [15]. After 30 min, the diameter of the hydrolysis zone was measured, and the experiments were carried out in triplicate.

Extraction of crude enzyme and fungal biomass determination

After incubation, the culture broth was filtered using filter paper (Whatman No. 1) to separate the fungal biomass. The filtrate containing the crude enzyme was then assayed for polygalacturonase activity. The filter paper with fungal biomass was dried at 80 °C until a constant weight was achieved, and the fungal growth was obtained by deducting the weight of the filter paper and the growth was then expressed as g/L [14]. All the experiments were performed in triplicate.

Polygalacturonase activity

The dinitrosalicylic acid (DNS) method was employed to determine polygalacturonase activity by measuring reducing sugars released from the hydrolyzation of pectin [16]. A 0.5 mL crude enzyme was added to a solution containing 0.5 mL of pectin (1 %) in 0.1 M citrate buffer, pH 4.5. After 30 min of incubation at 45 °C, a 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 a UV-visible spectrophotometer (Spectronic Unicam), and the galacturonic acid was set as a reference. The pectinase activity was expressed in terms of Unit (U). One unit of pectinase activity was defined as the amount of

pectinase that catalyzes the release of 1 µmol of galacturonic acid per mL of culture filtrate per minute under assay conditions.

Improvement of cultural conditions and medium compositions for polygalacturonase production

To enhance polygalacturonase production, various physical and chemical parameters [14] were determined including cultivation time (14 days with 24 h interval), temperature (20, 30, 37, 40 and 50 °C), initial pH (2.0, 4.0, 4.5, 5.0, 6.0 and 8.0), agitation speed (0, 50, 100, 150, 200 and 250 rpm), and inoculums sizes (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 spores/mL), carbon sources (carboxy methyl cellulose (CMC) fructose, glucose, lactose, pectin, starch and sucrose), pectin at different concentration (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 %; w/v), inorganic and organic nitrogen sources (ammonium chloride, ammonium nitrate, ammonium sulphate, sodium nitrate, peptone, urea, yeast extract) and yeast extract at different concentration (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 %; w/v). All the experiments were performed in triplicate.

Statistical analysis

One-way analysis of variance (ANOVA) and Duncan multiple range tests (DMRT) with PASW (SPSS) Statistics version 12.0 were performed to analyze the significant difference in the mean of experimental data. A 0.05 confidence level or $\alpha = 0.05$ was used to test all the experimental data.

Results and discussion

Isolation, screening, and identification of polygalacturonase producer

A total of 34 fungal isolates were isolated from rotten fruits with the highest number of fungal isolates (13 isolates) obtained from rotten oranges. Besides, 10 and 5 fungal isolates were obtained from rotten lemon and mango, respectively. Three isolates were successfully isolated from decayed papaya and banana (**Table 1**). The present study revealed fungi highly colonized citrus fruits due to the high amount of pectin. A previous study reported that orange and other citrus peels contain much more pectin compared to other fruits with citrus peels comprised of 30 % of pectin and commercial pectin is mainly extracted from citrus peels [17]. Out of 34, 70.6 % (24 fungal isolates) were able to secrete polygalacturonase based on the result of the hydrolysis zone through the pectinase screening medium (**Figure 1**). The result also revealed that 9 fungal strains isolated from lemon exhibited polygalacturonase activity with a diameter inhibition zone between 10.0 ± 0.07 - 23.7 ± 0.11 mm. Meanwhile, 11 out of 13 fungal isolates from rotten orange showed polygalacturonase activity with the diameter of the hydrolysis zone ranging from 13.3 ± 0.04 to 45.3 ± 0.04 mm. Only 5 fungal strains isolated from mango demonstrated pectinase activity with a diameter of hydrolysis zone in the range of 16.3 ± 0.04 - 26.7 ± 0.04 mm. However, none of the fungal strains isolated from banana and papaya exhibited polygalacturonase activity. Since fungal strain SO5 showed the highest polygalacturonase activity with a diameter zone of 45.3 ± 0.04 mm (**Figure 2(a)**), it was chosen for further study. Based on morphological observation through a light microscope and macro morphologies (result not shown), the fungal strain SO5 was identified as *Aspergillus* spp. based on the structure of conidia, conidiophores, vesicle, medullae, and phialides. This isolate has radiated conidial heads with a diameter of 30 - 40 µm. The conidiophores wall of the strain was thick with a diameter of approximately 1.78 µm and a width value of 11.54 µm. Besides, the conidiophores were smooth and colourless to slightly brown in colour, especially near apices with approximately 466.89 µm long. The vesicle of the fungal strain was nearly spherical, brownish to black in colour, and 46.63 µm wide. Furthermore, the fungal vesicle was biseriate with metulae covering virtually the entire surface of the vesicle and the formation of a single septum called phialides. **Figure 2(b)** shows the morphology of *Aspergillus* spp. SO5 under a light microscope with the formation of vesicle, conidia, conidiophore, and phialides. Several previous studies have demonstrated the significance of *Aspergillus* spp. as a promising strain for fungal polygalacturonase. For instance, Doughari and Onyebarachi [18] reported *Aspergillus flavus* grown on orange peels showed significant polygalacturonase production.

Table 1 Number of fungal colonies isolated from various rotten fruits and their polygalacturonase production.

No	Sources	Number of fungal colonies isolated	Number of isolates showed polygalacturonase activity
1	Lemon	10	9
2	Mango	5	4
3	Orange	13	11
4	Papaya	3	-
5	Banana	3	-
Total		34	24

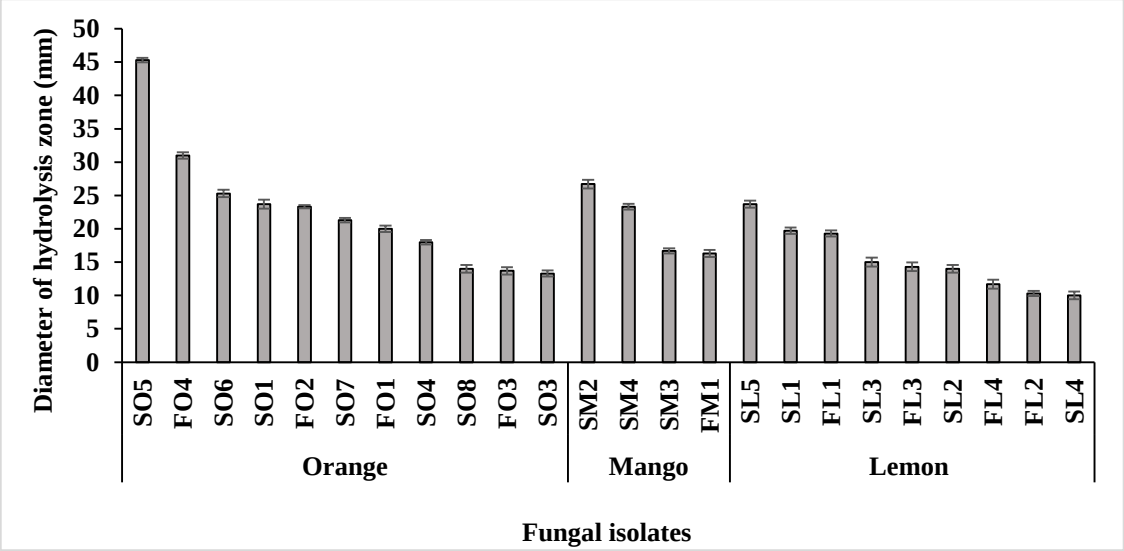

Figure 1 Diameter of hydrolysis zone produced by fungal isolates.

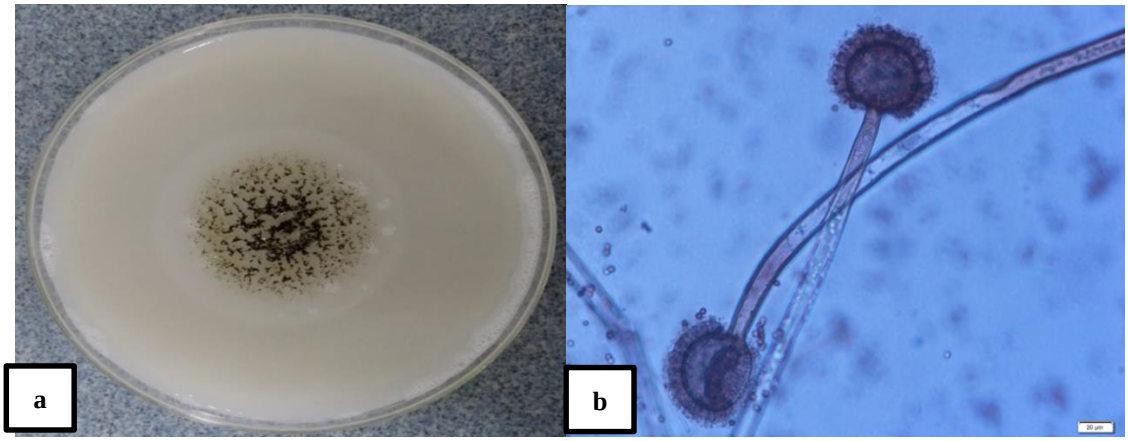

Figure 2 Polygalacturonase producer screening and identification of fungal isolate SO5. (a) Primary screening on pectin agar (b) Light microscopy observation of *Aspergillus* sp. SO5 at magnification 20X.

Time course profile of polygalacturonase activity before the enhancement of physicochemical parameters

Figure 3 shows the time-course profile of polygalacturonase activity from *Aspergillus* sp. SO5 before improved cultural conditions and chemical compositions. It was observed that the highest polygalacturonase production was achieved on the 6th day of cultivation with a value of 8.68 ± 0.13 U/mL and the fungal growth of 0.66 ± 0.02 g/L. The production of enzymes increased over time from day 0 and peaked at day 6 of the incubation period. Similarly, the fungal growth also endures the same pattern, and it was concluded that maximum fungal biomass and enzyme production is dependent. However, the enzyme production declined beyond the maximal level, which may be due to variation of pH in the culture medium due to the formation of organic acids and hydrolyzing of the enzyme by the fungus for biomass protein synthesis after a certain time [19]. Generally, cultivation time varies depending on fungal strains and the type of fermentation. For instance, Ire and Vinking [20] reported that the highest polygalacturonase production by *Aspergillus niger* grown on banana peel under submerged and solid-state fermentation was achieved on day 2 and day 3 of cultivation, respectively. Meanwhile, fungal isolate *Trichoderma harzianum* achieved its maximum polygalacturonase production on day 5 of incubation under submerged fermentation [12].

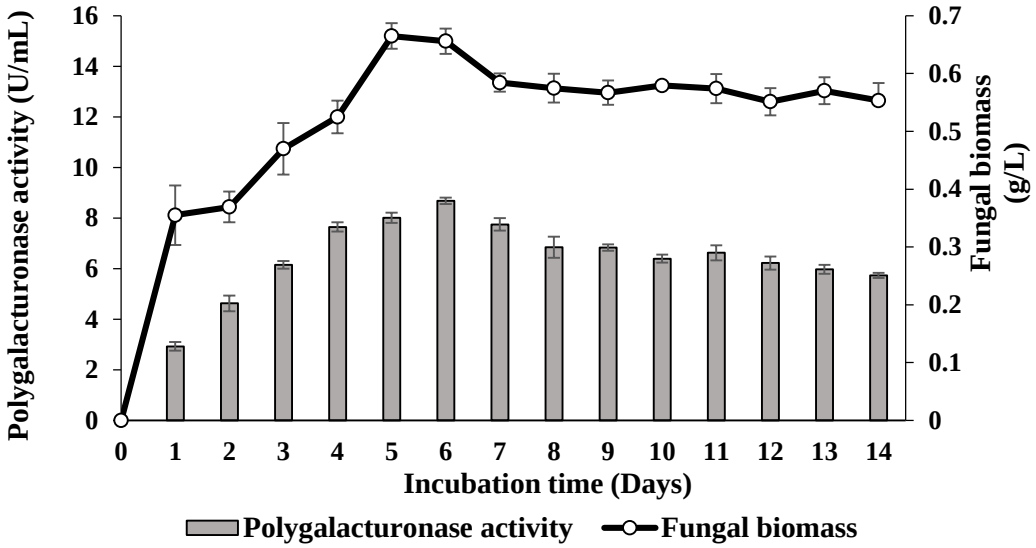

Figure 3 Time-course profile of polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5 before improved physicochemical parameters.

Effect of pH

In the present study, the initial pH of the culture medium was found to have a remarkable effect on polygalacturonase production. The finding revealed that pH 4.5 (the samples were taken on the 6th day) exhibited maximal enzyme activity of 8.71 ± 0.15 U/mL (**Figure 4**). A similar observation was reported by Sharma and Rishishwar [21] who found that pH 4.5 was the optimum pH for maximum polygalacturonase activity by *Aspergillus niger*. It was noteworthy that pH 4 to pH 6 demonstrates fair polygalacturonase activity. This may be due to the fungal strain that has been isolated from citrus fruit which is acidic in nature and the strain can tolerate an acidic medium. Besides, the polygalacturonase production declined with an increase in pH medium. It was suggested that the enzyme of *Aspergillus* sp. SO5 was inactive in an alkaline medium and thus led to lower polygalacturonase activity. According to Fontana and Silveira [22], the pH of the culture medium for fungal cultivation is influenced by the absorption of nitrogen source, formation, and composition of organic acids, and the release of hydrogen (H^+) ions. Thus, any instability of the pH medium could lead to low enzyme production.

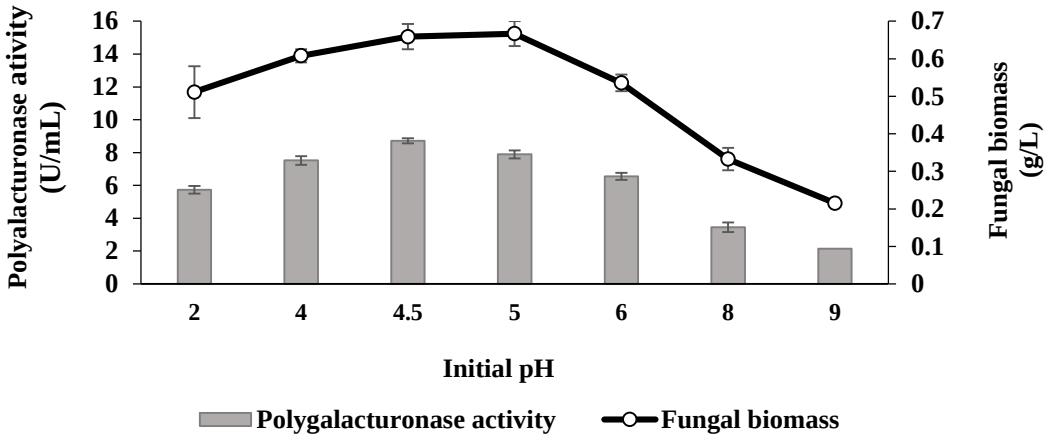

Figure 4 Effect of initial pH of culture medium on polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5.

Effect of inoculum size

Inoculum size is another crucial factor for polygalacturonase production. Different inoculum density ranges 1×10^4 - 1×10^8 spores/mL were optimized for better polygalacturonase production and the result was tabulated in **Figure 5**. It was found that the inoculum size of 1×10^6 spores/mL exhibited the highest polygalacturonase activity with a value of 9.60 ± 0.11 U/mL (the samples were taken on the 6th day). Anuradha [23] reported a similar result, finding that 1×10^6 spores/mL was the optimum inoculum density for maximum polygalacturonase production by *Aspergillus awamori* MTCC 9166. The finding also revealed a lesser and higher inoculum density than the optimum level resulting in lower polygalacturonase activity. Low inoculum density might be insufficient for initiating fungal growth since an adequate spore number ensures rapid proliferation of biomass and enzyme synthesis. In contrast, excessive spore density resulted in a depletion of nutrients [14]. Besides, the drop in pectinase production might be due to the instability of enzymes at low or high pH since the protein is generally denatured at the extreme pH value of the culture medium.

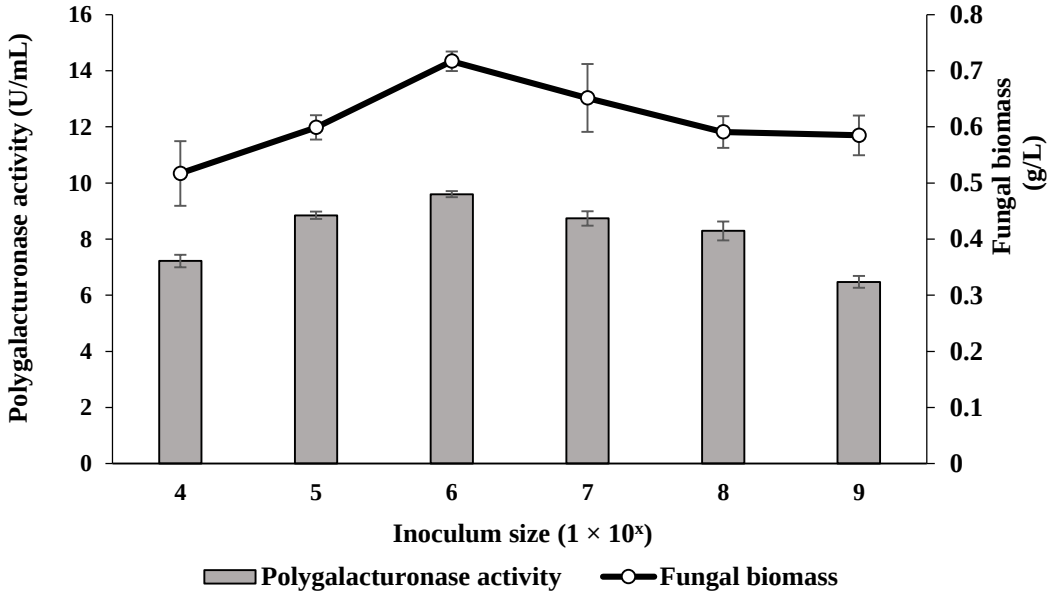

Figure 5 Effect of inoculum size on polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5.

Effect of agitation speed

The effect of agitation speed on enzyme production is depicted in **Figure 6**. The finding revealed that polygalacturonase activity increased with an increase in agitation speed up to 100 rpm (the samples were taken on the 6th day) and the activity declined thereafter. The finding demonstrated the optimum production of polygalacturonase occurred when the agitation speed was 100 rpm with a value of 15.59 ± 0.22 U/mL. The previous studies have reported various optimum agitation speeds might be due to differences in fungal strains and culture conditions. For instance, Palaniyappan [24] reported the optimum agitation speed for the highest polygalacturonase production by *Aspergillus niger* MTCC 281 under submerged fermentation was 170 rpm. Higher agitation speed (250 rpm) strongly suppressed the enzyme production with only 6.16 ± 0.18 U/mL observed. According to Darah [25], higher agitation speed resulted in shear forces and damage to the microbial cells and thus, harming enzyme production. Meanwhile, lower agitation speed than the optimal value resulted in less polygalacturonase activity, which could be due to aeration, inadequate dissolved oxygen, and nutrient transfer rate. Darah [26] reported mixing the culture medium is important for the assimilation of sugars, oxygen, and nutrient transfer rate which enhances the microbial synthesis of enzymes. Thus, inadequate agitation speed resulted in low dissolved oxygen and nutrient transfer, leading to low enzyme production.

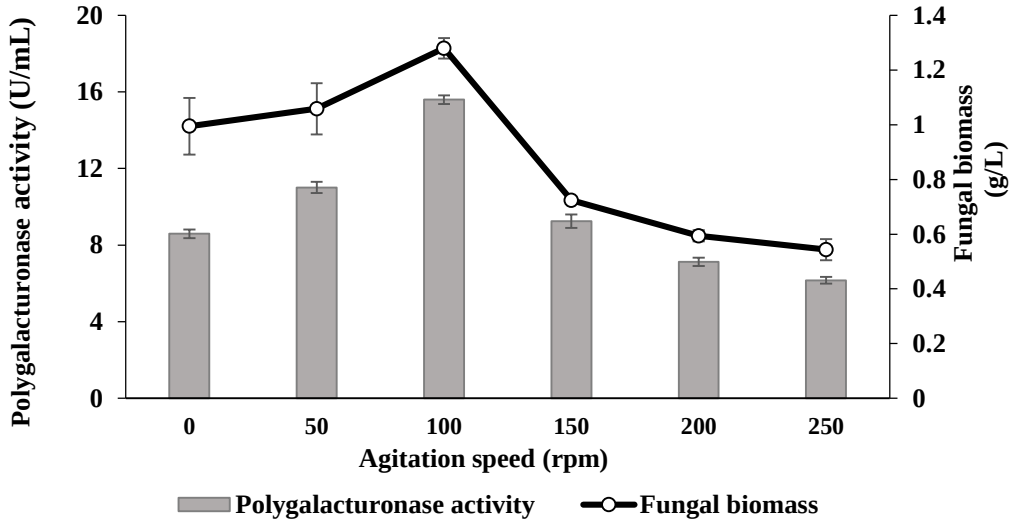

Figure 6 Effect of agitation speed on polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5.

Effect of incubation temperature

Of the various range of temperatures studied, an incubation temperature of 30 °C was found to support maximal polygalacturonase production with the value of 15.42 ± 0.19 U/mL (the samples were taken on the 6th day) and the fungal growth of 1.29 ± 0.05 g/L (**Figure 7**). The finding was in line with Batool [27] who found that 30 °C was the optimum temperature for maximal pectinase production and fungal growth from *Aspergillus niger*. It was observed that incubation temperature beyond the maximum level leads to a decrement in polygalacturonase production and fungal growth. Besides, the finding also revealed the lower temperature (20 °C) significantly suppressed the enzyme production and fungal biomass with only 8.12 ± 0.09 U/mL and 0.86 ± 0.01 g/L observed, respectively. According to Abdullah [28], a higher temperature may lead to denaturation of an enzyme, and a lower temperature may slow fungal growth, and thus, result in less enzyme production. The optimum temperature may vary depending on fungal species. This phenomenon may be due to genetic differences in various fungal strains [29]. For instance, Sandri [30] found the optimum temperature of exo-polygalacturonase production by *Aspergillus fumigatus* was 60 °C, whereas for endo-polygalacturonase in between intervals of 40 - 60 °C. Meanwhile, Ketipally and Ram [10] reported the maximum polygalacturonase production by *Aspergillus oryzae* RR103 was achieved at an incubation temperature of 35 °C.

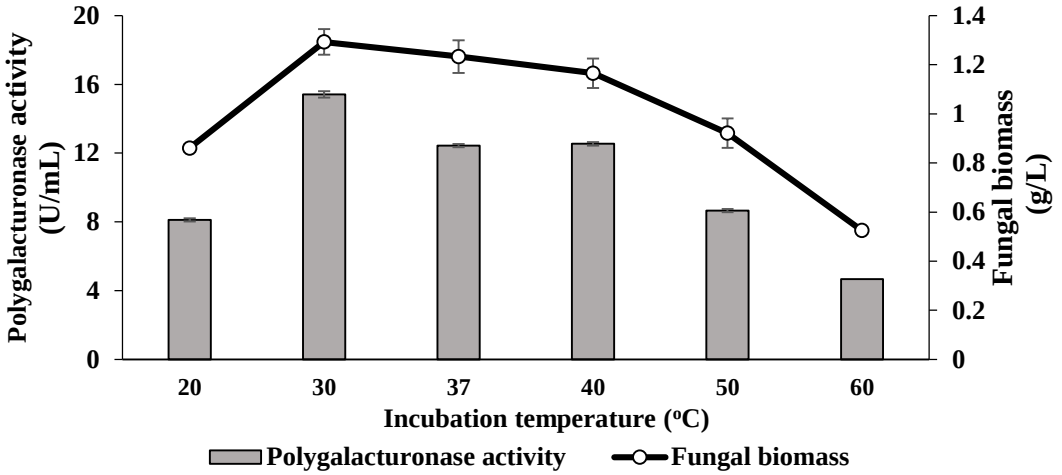

Figure 7 Effect of incubation temperatures on polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5.

Effect of carbon sources

Carbon sources at a concentration of 0.4 % were incorporated separately in the basal medium. The effect of these carbon sources was determined, and the results are illustrated in **Figure 8**. Pectin was found to be stimulating the polygalacturonase activity with a value of 16.93 ± 0.25 U/mL and fungal biomass was 1.37 ± 0.15 g/L (the samples were taken on the 6th day). Jayani [31] reported a similar observation, finding that citrus pectin had the highest production of polygalacturonase. The present finding was in agreement with a previous study that reported a combination between pectin and yeast extract in a culture medium to be a good substrate in stimulating pectinase production [32]. The effect of pectin as the best carbon source was further studied at different concentration levels and the result is illustrated in **Figure 9**. It was observed that the polygalacturonase activity increased with an increased concentration of pectin. Pectin supported the highest enzyme production at a concentration of 0.5 and 0.6 % with a value of 22.74 ± 0.18 U/mL and 22.62 ± 0.60 U/mL, respectively. The fungal growth and enzyme activity slightly declined beyond the optimal concentration level. This might be due to the accumulation of galacturonic acid due to excessive pectin degradation [14] which could be toxic to microbial cells. Similarly, Sudeep [33] observed that 1 % of substrate resulted in maximum pectinase production and substrate concentration beyond the optimal level decreased the enzyme production. They also suggested that excessive substrates and nutrients in the culture medium inhibited microbial growth and lowered enzyme activity.

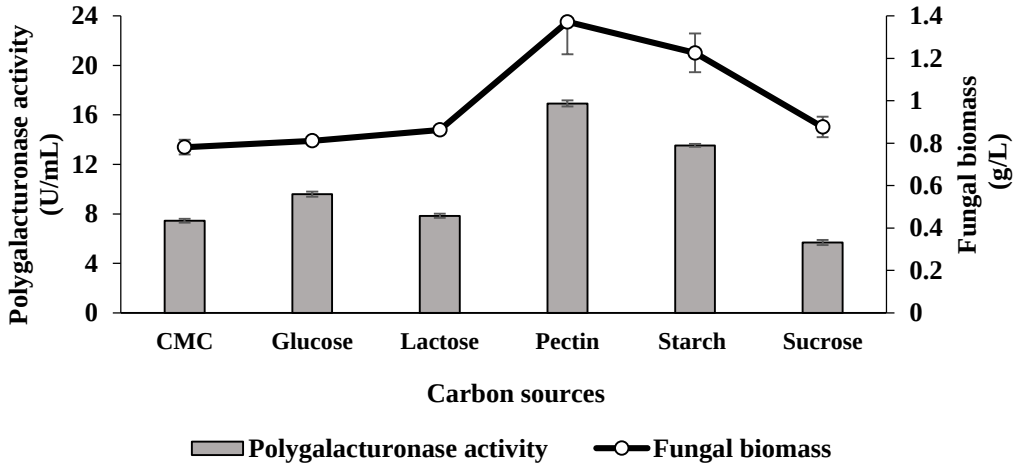

Figure 8 Effect of carbon sources on polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5.

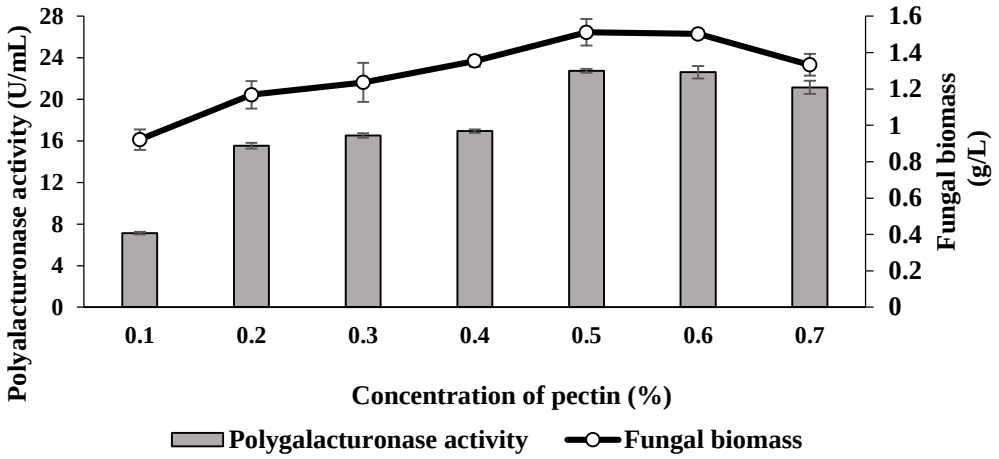

Figure 9 Effect of pectin concentration on polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5.

Effect of nitrogen sources

Different organic and inorganic sources were tested for maximal production of polygalacturonase. Maximum polygalacturonase production (24.42 ± 0.30 U/mL) was observed when yeast extract was incorporated into the culture medium as a nitrogen source (**Figure 10**). Yeast extract has been reported to enhance the maximum polygalacturonase production from *Aspergillus niger* BC23 [34]. This might be due to the role of yeast extract as essential substances provider including amino acids, minerals, and vitamins that are crucial to support microbial growth and enzyme production as well [35]. It was observed that inorganic nitrogen sources including ammonium nitrate, ammonium sulfate, and sodium nitrate slightly suppressed enzyme production compared to organic nitrogen sources. A similar observation was reported by Abdullah [28] and they suggested that the organic nitrogen sources were better growth stimulators compared to inorganic sources. **Figure 11** demonstrates the effect of yeast extract as a nitrogen source at a different concentration level. It was observed that the highest enzyme production was achieved when the culture medium was supplemented with 0.3 and 0.4 % with a value of 32.05 ± 0.45 U/mL and 32.04 ± 0.51 U/mL, respectively. A higher concentration than the optimal value leads to the decrement of enzyme production. The excessive nitrogen sources might be influenced by the pH of the culture medium and the imbalance of pH harmed enzyme production.

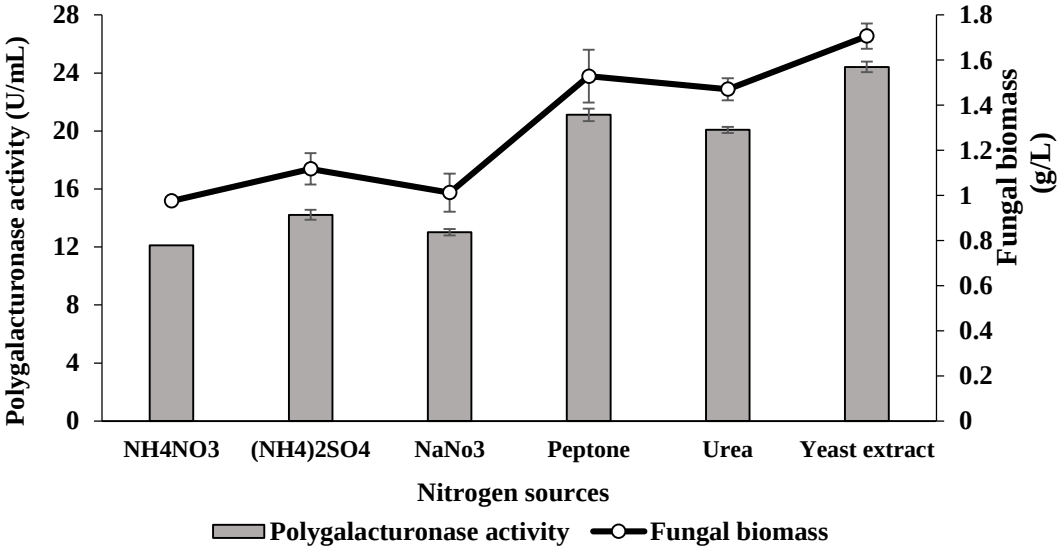

Figure 10 Effect of nitrogen sources on polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5.

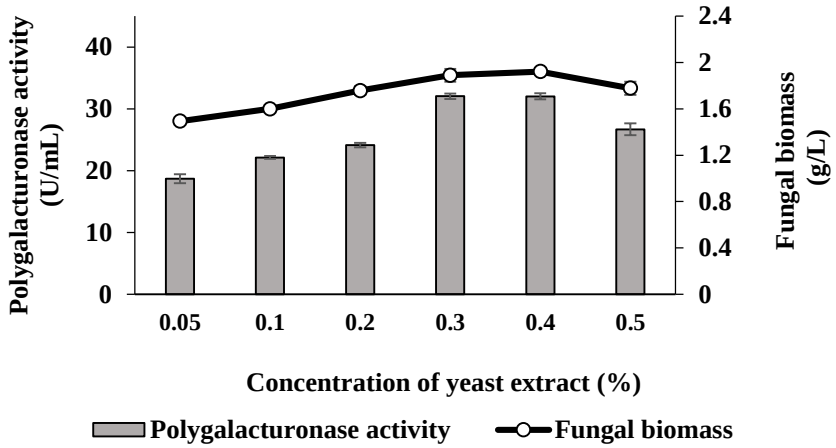

Figure 11 Effect of yeast extract concentration on polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5.

Time course profile of polygalacturonase activity after enhancement of physicochemical parameters

All optimized conditions were incorporated and the time-course profile of polygalacturonase activity was determined. The present study demonstrated that maximum polygalacturonase production was observed after 5 days of cultivation with a value of 34.21 ± 0.49 U/mL and the enzyme activity decreased thereafter (**Figure 12**). The reduction of polygalacturonase activity after achieving maximum production may be due to the depletion of the available nutrients such as carbon sources. Besides, any further increase in the incubation period leads to lower enzyme production since the fermentation process depends on microbial strains, the fermentation medium's nature, the physiology conditions process, and the concentration of nutrients [36]. It was summarized that the improvement of physiochemical parameters including inoculum density, agitation speed, initial pH, carbon source and its concentration, and nitrogen source with its concentration significantly enhanced the production of polygalacturonase. The polygalacturonase activity and fungal biomass increased up to 74.63 and 69.44 %, respectively **(Table 2**). The enzyme production before and after improved physiochemical parameters were 8.68 ± 0.13 and 34.21 ± 0.49 U/mL, respectively. Meanwhile, the fungal biomass was 0.66 ± 0.02 g/mL before improvement, and its growth increased by approximately 2.16 ± 0.07 g/mL after improvement. It was noteworthy that the incubation period was shortened to day 5 compared to before enhancing physicochemical parameters (6 days). Thus, the shorter incubation period will make this fungal strain cost-effective for commercial exploitation.

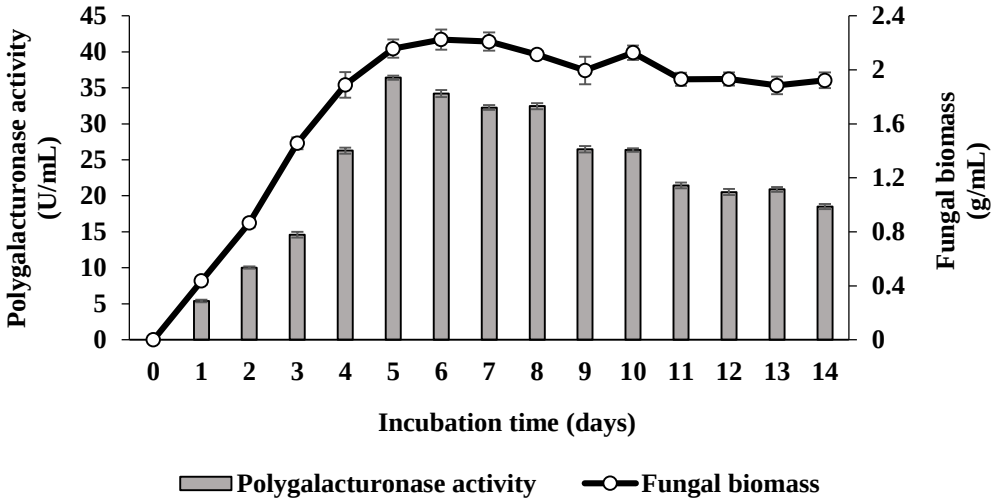

Figure 12 Time-course profile of polygalacturonase activity and fungal growth of *Aspergillus* sp. SO5 after improved physicochemical parameters.

Table 2 The summary of polygalacturonase production and fungal growth by *Aspergillus* sp. SO5 after improvement of physicochemical parameters in a shake flask system.

Parameters	Profiling before improvement	Profiling after improvement
Incubation time (days)	6	5
Initial pH	4.5	4.5
Inoculum size (spores/mL)	1×10^7	1×10^6
Agitation speed (rpm)	150	100
Incubation temperature (°C)	30	30
Carbon sources	pectin	pectin
Concentration of pectin (%)	0.4	0.5
Nitrogen sources	none	yeast extract
Concentration of NH_4NO_3	0.2	0.3
Polygalacturonase activity (U/mL)	8.68 ± 0.13	34.21 ± 0.49
Increment (%)	-	74.63
Fungal growth (g/L)	0.66 ± 0.02	2.16 ± 0.07
Increment (%)	-	69.44

Conclusions

The present study revealed that the rotten citrus fruits were highly colonized with fungal pectinase producers since 70.6 % of isolated fungi are able to produce polygalacturonase. *Aspergillus* sp. SO5, a fungal strain isolated from rotten orange exhibited a significant polygalacturonase producer with a hydrolysis zone of 45.3 ± 0.04 mm. Besides, the improved physiochemical parameters including incubation temperature, initial pH, agitation speed, inoculum density, carbon, and nitrogen sources have significantly enhanced polygalacturonase production by up to 74.63 % (34.21 ± 0.49 U/mL). It was observed that the improved physicochemical conditions could enhance polygalacturonase production and shorten the incubation period from 6 days to 5 days. Further studies are necessary especially on purification and characterization of the enzyme and identifying potential isolate to a species level since the present findings revealed the potential of *Aspergillus* sp. as a polygalacturonase producer.

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