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Isolation and Screening of Pectinolytic Fungi from Orange (*Citrus nobilis* Tan.) and Banana (*Musa acuminata* L.) Fruit Peel

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Abstract. Pectinase is the one of most important enzyme which is used in food industry such as fruit and vegetable juice extraction, oil extraction and fermentation of coffee, cocoa and tea. Pectinase can be produced by microorganism such as bacteria and fungi. Fungi are known as potent producer of pectinase. This research was conducted to isolate and screen of the pectinolytic fungi from rotten orange and banana fruit peels. This research succeeded to isolate 10 fungal isolates from rotten orange peels and 5 fungal isolates from rotten banana peels. These isolates were screened in pectinolytic activities based on clear zone formation on pectic medium which is stained by cetyl trimethyl ammonium bromide. The screening result showed that fungal isolates which showed pectinolytic activity were O2, O3, O4, O7, O8, O10, B3, and B5. Based on morphological characters, pectinolytic fungi were identified as *Fusarium* O4 and O10, *Penicillium* O2, *Aspergillus* O3, O7, B3 and B5 and *Trichoderma* O8. The highest pectinolytic activity was showed by *Penicillium* O2 which was isolated from orange peel.

1. Introduction

Pectinase is a group of enzymes that catalyze a degradation of pectin through depolymeration (hydrolases and lyases) and de-esterification (esterases) reactions [1]. Based on their mode of action, the pectinase is classified into three types: pectin esterase, hydrolases and lyases. Pectin esterase catalyze the de-esterification of the methoxyl group of pectins, forming pectic acid. Hydrolases (polygalacturonases and polymethylgalacturonases) catalyze the hydrolytic cleavage of α-1,4-glycosidic linkage in pectic acid and pectin. Lyases (polygalacturonate lyase and polymethylgalacturonate lyase) catalyze the cleavage of α -1,4-glycosidic linkage in pectic acid and pectin, forming unsaturated galacturonates and methyl galacturonates [2].

Pectinases share about 25% in global sales of food enzymes [3]. Pectinases are widely used in food industries, such as fruit processing industry for extraction and clarification of fruit juice, preparation of fruit cordials [4], enhance process efficiency of wine making and wine quality [5], oil extraction and fermentation of coffee, cocoa and tea [3]. Since pectinases are widely used enzyme for different industrial application, it is necessary to improve pectinase production, including selection for potential source of pectinase. Many experiments have attempted to increase the performance of enzyme production [6]. The new microbes with high extracellular pectinase activity, stability over wide range of temperature and pH for a longer period of time, with their cost-effective production have been the focus of recent research [2].

Nowadays, commercial pectinase preparations are obtained from fungi [5]. Fungal pectinases are commonly used in fruit industry because fungi are potent producers of pectic enzymes and the optimal pH of fungal enzymes are very close to the pH of many fruit juices, in the pH range of 3 to 6 [7]. This research was conducted to isolate and screen of pectinolytic fungi from orange and banana fruit peels.

2. Methodology

2.1. Medium

This research used modified pectic agar medium to isolate and screen a pectinolytic fungi [8]. The composition of modified pectin agar medium (g/l, wt/vol.) were: Pectin 5.00, K_2HPO_4 0.50, $MgSO_4.7H_2O$ 0.10, NaCl 0.20, $CaCl_2.2H_2O$ 0.20, $FeCl_3.6H_2O$ 0.01, Yeast extract 1.00, Agar 20.00.

2.2. Isolation and Screening of Pectinolytic Fungi

The fungi were isolated from rotten orange fruit peels collected in Jember East Java and rotten banana fruit peels collected in Lumajang East Java. A slice of rotten peels was put on pectic agar medium and had been incubated for 2-3 days. Whereas, the different fungal colonies were inoculated on potato dextrose agar (PDA) for purification. The spores of pure culture were maintained in PDA medium in test tubes sealed with parafilm and stored at 4 °C for further use.

The screening of the pectinolytic activity was conducted based on a clear zone formation on pectic agar medium after it was stained by Cetyl trimethyl ammonium bromide. Fungal isolates were inoculated on pectic agar medium and had been incubated at a room temperature for 48 hours. .

2.3. Identification of Pectinolytic Fungi

Pectinolytic fungi were identified based on macroscopic and microscopic morphological characters. These characters were compared to Pictorial Atlas of Soil and Seed Fungi, Second Edition [9] until genus level.

3. Result and Discussion

3.1. Isolation and Screening of Pectinolytic Fungi

This research succeeded to isolate 10 fungal isolates from rotten orange peels named as isolate O1 until O10, 5 fungal isolates from rotten banana peels named as B1 until B5. These isolates were screened in pectinolytic activities based on clear zone formation on pectic agar medium which is stained by cetyl trimethyl ammonium bromide also known as cetrimide or CTAB. The screening result showed that fungal isolates which showed pectinolytic activity were O2, O3, O4, O7, O8, O10, B3, and B5. The diameter of clearing zone of each pectinolytic isolates are shown in table 1. Among them, isolate O2 showed the highest pectinase activity.

Table 1. Pectinolytic activity of fungal isolate showed by clear zone formation on pectin medium after incubation at room temperature for 48 hours.

Code of Isolate	Clear Zone Diametre (mm)
O2	3.44
O3	1.14
O4	1.42
O7	1.00
O8	2.30
O10	1.46
B3	0.88
B5	1.05

This research showed that the rotten orange peels was a more potential source of pectinolytic fungi than the rotten banana peels. Citrus peels especially orange peels are a rich source of pectin since it contains the most microbes such as bacteria and fungi. Orange peels have been reported to contain 18.3% pectin and is utilized for commercial extraction of pectin [10].

Pectinolytic isolates that depolymerized pectin produced colourless hydrolytic zones around colonies on pectic agar medium after colored by CTAB (Figure 1). The cetrimide or CTAB is an insoluble acidic precipitant of polysaccharide. Enzymatic hydrolysis of the pectic substrates inhibited their precipitation by using CTAB, which then lead to the appearance of cleared zones in front of the pectin hydrolases and lyases [11].

Figure 1. Isolate O2 showing clear zone on pectic agar medium. Clear zone formation indicating a pectinolytic activity.

3.2. Identification of Pectinolytic Fungi

Based on morphological characters, pectinolytic fungi was identified as belonging to 4 genera, i.e. *Penicillium* (O2), *Aspergillus* (O3, O7, B3, B5), *Fusarium* (O4, O10) and *Trichoderma* (O8) (table 2).

Table 2. Identification result of pectinolytic fungi isolated from Orange and Banana peels based on morphologycal characters.

Code of Isolate	Genera
O2	*Penicillium*
O3, O7, B3, B5	*Aspergillus*
O4, O10	*Fusarium*
O8	*Trichoderma*

3.2.1. Penicillium

Isolate O2 which had highest pectinolytic activity was identified as belonging to the genera of *Penicillium*. Morphologycal characters of isolate O2 is showed on figure 2. This isolate produced white mycelium with dark green conidia. Whereas, a branched conidiophore showed a brush-like apearance and condiophore which was end with bottle shaped phialides. This microscopic character is a great taxonomic importance of *Penicillium* [12]. *Penicillium* have been known as pectinase producer, i.e. *Penicillium chrysogenum* [13], *Penicillium citrinum* [8].

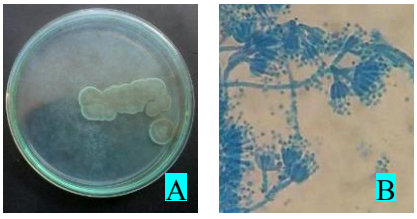

Figure 2. *Penicillium* O2 on PDA, 6 days old (A) and brush-like conidiophores (B).

3.2.2. Aspergillus

Isolates O3, O7, B3 and B5 were identified as belonging to the genera of *Aspergillus*. Morphological characters of these isolates are showed in figure 3. These isolates showed the same microscopic characters, conidiophores upright, simple, terminating in vesicles bearing philiades. These characters were great important taxonomic characteristics for *Aspergillus* [9]. Isolate O3 and B3 had the same macroscopic characters, formed white mycelium with radial zonation, black conidia and yellow pigmentation on the center of reverse site. Isolate O7 and B5 formed white yellowish mycellium with yellow greenish conidia. *Aspergillus* is one of pectinolytic fungi that often produce acid pectinases used in food processing industry [1]. Pectinolytic thermophilic fungi were isolated from soil, vegetables and fruit waste. The most potential isolate as pectinase producer was reported as *Aspergillus fumigatus* which produce thermophilic pectinases that active in low pH [14].

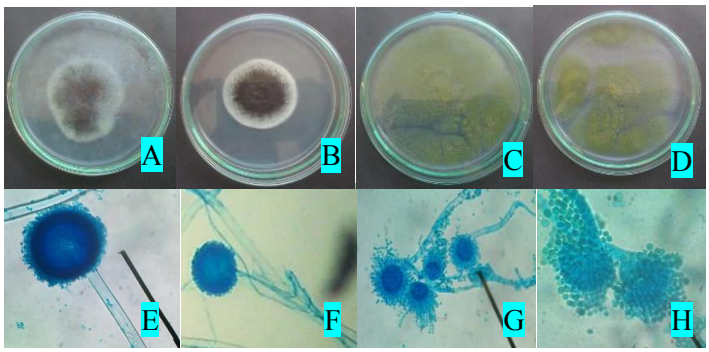

Figure 3. Colonies of *Aspergillus* strains on PDA plate, 6 days old. *Aspergillus* O3 (A) and B3 (B) showed black conidia. *Aspergillus* O7 (C) and B5 (D) showed yellow greenish conidia. These isolates formed conidiophores terminating in vesicles (E,F,G,H).

3.2.3. Fusarium

Isolates O4 and O10 were identified as *Fusarium.* Morphological characters of these isolates are showed in figure 4. *Fusarium* O4 produced white mycelia with yellow pigmentation on the center of the colony. *Fusarium* O10 produced mycelium with pink pigmentation (dorsal and reverse). *Fusarium* O4 and O10 produced lunar shape macroconidia with septation. These characteristics were regarded as taxonomically useful characteristics for *Fusarium* [9]. Shorter macroconidia was shown by *Fusarium* O10. *Fusarium* is known as pectinase producer. Strong activity of pectinase was detected from F. *oxysporum* and F. *proliferatum* [15].

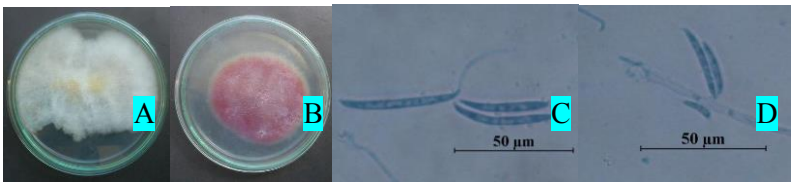

Figure 4. Colonies of *Fusarium* strains on PDA plate, 6 days old (top view), *Fusarium* O4 (A) and O10 (B) and macroconidia produced by *Fusarium* O4 (C) and O10 (D).

3.2.4. Trichoderma.

Isolate O8 was identified as *Trichoderma*. Morphological characters of isolate O8 was showed in figure 5. This isolate formed white mycelium with grey conidia, branched conidiophores and elipsoidal conidia. The colour of conidia was green when it puts under the light microscopes. These characters were regarded as taxonomically useful characteristics of *Trichoderma* [9]. The production

of notable, safe and highly active pectinase of *Trichoderma* was reported [16]. This enzyme was used in fruit juices clarification with remarkable stability.

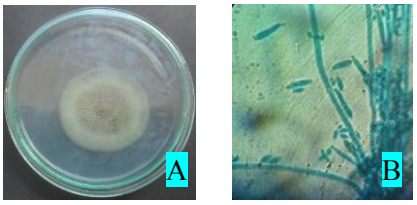

Figure 5. Colony of *Trichoderma* O8 on PDA plate, 6 days old (A) and micro morphological view (B).

4. Conclusion

The Total of 15 fungal isolates were obtained in this research consist of 10 isolates from Orange peel and 5 isolates from Banana peel. Fungal isolates which showed pectinolytic activity were O2, O3, O4, O7, O8, O10, B3, and B5. Based on morphological characters, pectinolitic fungi were identified as *Penicillium* O2, *Aspergillus* O3, O7, B3 and B5, *Fusarium* O4 and O10, and *Trichoderma* O8. The highest pectinolytic activity was showed by *Penicillium* O2 which was isolated from rotten orange peel.

References

[1] Pedrolli D B, Monteiro A C, Gomez E, and Carmona E C 2009 *The Open Biotech J.* **3** 9-13
[2] Garg G, Singh A, Kaur A, Singh R, Kaur J, and Mahajan R 2016 *Biotech* **6** 47
[3] Sieiro C, Gracia-Fraga B, Lopez-Seijaz J, da Silva A F, and Villa T G 2012 *Food Industrial Processes-Metodes and Equipment www.intechopen.com*
[4] Tapre A R, and Jain R K 2014 *Int Fd Res J.* **21(2)** 447-453
[5] Merín G M, Martín M C, Rantsiou K, Cocolin L, and Morata de Ambrosini V I 2015 *Braz J. Microbiol* **46(3)** 815-823
[6] Molina S M W, Pelissari F A, Vitorello C B M 2001 *Braz J. Microbiol* **32(4)** 320-326.
[7] Jayani R S, Shukla S K, and Gupta R 2010 *Enzime Res* **31** 306785
[8] Sandhya R, and Kurup G 2013 *Int Res J. Biol Sci* **2(9)** 34-3
[9] Watanabe T 2002 *Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species*
[10] Jabeen A, Hanif Q A, Hussain M, Munawar A, Farooq N, and Bano S 2015 Sci Lett **3(2)** 42-45
[11] Hadj-Taieb N, Tounsi H, Chabchoub A, Abid N and Gargouri A 2011. *Appl Biochem Biotechnol* **165**:1652–1660
[12] Visagie C M, Houbraken J, Frisvad J C, Hong S.-B, Klaassen C H W, Perrone G, Seifert K A, Varga J, Yaguchi T, and Samson R A 2014 *Studies in Mycology* **78** 343–371
[13] Banu A R, Devi M K, Gnanaprabhal G R, Pradeep B V, and Palaniswamy M. 2010 *Indian J. Sci. Technol.* **3(4)** 377-381
[14] Phutela U, Vikram D, Shobhna S, and Chadha B S 2005 *Braz J. Microbiol* **36** 63-69
[15] Kwon H W, Yoon J H, Kim S H, Hong S B, Cheon Y, and Ko S J 2007 *Mycobiology* **35(3)** 162–165
[16] Ismail A-M S, Abo-Elmagd H I, and Housseuny M M 2016 *J.Genet Eng Biotechnol* **14** 153-159