ISSN No. 2087-4855

Vol. 2 No. 1 - April 2011

JURNAL HORTIKULTURA INDONESIA

(Indonesian Journal of Horticulture)

Penerbit
Perhimpunan Hortikultura Indonesia
(Indonesian Society for Horticulture)

Jurnal Hortikultura Indonesia

(Indonesian Journal of Horticulture)

ISSN : 2087-4855

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Jurnal Hortikultura Indonesia merupakan jurnal yang menyajikan hasil penelitian, analisis kebijakan dan review yang berhubungan dengan bidang hortikultura. Jurnal diterbitkan setahun tiga kali : April, Agustus, dan Desember

No Rekening Berlangganan :

Rekening a/n	: PERHIMPUNAN HORTIKULTURA INDONESIA
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J. Hort. Indonesia 2(1):43-51. April 2011.

Exploration of Pectin – Utilizing Yeast From Soil of Bogor and Wleri Fruit Orchards

Fahrizal Hazra[1*]

Received 17 October 2010/Accepted 18 Maret 2011

ABSTRACT

There is a high demand on pectin–utilizing yeasts for industrial, agricultural and environmental purposes. Further exploration of yeast from various sources are important to enrich yeast culture collections. Nine yeast strains were isolated from various soil sources sampled based on biological sampling in Bogor and Central Java. Enriched media containing pectin as carbon sources was employed for isolation of the yeast. The isolated yeast were identified according to the methods described in monographs by Kreger – Van Rij (1984), Barnett et.al (2000), Guilliermond and Tanner (2006) . The strains isolated were taxonomically separated into 3 groups. Group I contains 3 strains, and this group is closely related to Candida tropicalis. Group II contains 4 strains, and this group is included in this genus Rhodotorula. Group III contain 2 strains, and this group is closely related to Williopsis saturnus, which is a synonym of Hansenula saturnus. Pectinolytic enzymes (Polygalacturonase) were produced by all of the tested strains. Polygalacturonase was produced as high as 1.7 U.ml^{-1} by strain no. 111 of group I, 1.7 U.ml^{-1} by strains no. 123 of group II, and 1.0 U.ml^{-1} by strain no. 211 of group III.

Key words : yeast, pectin, polygalacturonase

INTRODUCTION

Yeasts are eucaryotic microorganism, also may be defined as uni cellular fungi reproduced by budding or fission. Flagel (1977) has pointed the advantages of this morphological definition which leaves out others such as historical consideration discussed by Lodder and Kreger-Van Rij (1952). Budding yeast cells may be stage in the life cycle of multicellular fungi (Kreger-Van Rij, 1984). Yeasts are widely distributed in nature, can be found in the soil, water, fermented food, plant materials such as leaves and fruits, and also can be found in coastal water and sediments (Kimura *et.al.*, 1985).

Exploration of yeast from various sources is important to increase yeast collection (Hazra, 2005). Some workers tend to use available yeasts for their study. On the other hand, there is a high demand on yeasts for industrial, agricultural, and environmental purposes. Bacteria and fungi are commonly used by many workers to study pectolytic enzymes. However, studies on the enzymes production in yeast was quite limited. Some studies have been conducted from yeast of *Kluyveromyces fragilis* (Sakai *et al.*, 1984), from *Galactomyces reesei* (Sakai and Yoshitake, 1984), from *Trichosporon penicillatum* (Sakai *et al.*, 1982; Sakai and Okushima, 1982), from *Saccharomyces fragilis* (Lim *et al.*, 1980).

Isolated yeasts are likely to produce pectinolytic enzymes (Kaur *et al.*, 2004; Leizerson and Shimoni, 2005), as they are able to utilize pectin as a sole carbon source. Pectinolytic enzymes were reported to be produced extracellularly (Schomburg and Salzmann, 1991). Improved strains are important to be developed. For that purpose, it is necessary to increase various strains by appropriate methods such as isolation which is parallel to genetic engineering.

Isolates of yeasts were identified based on their morphology, physiology, and biochemical (Kreger-Van Rij, 1984; Barnett *et.al.*, 2000). The result can be compared with the taxonomic describtion of pectin or pectic substance utilizing yeast identified previously. In this study, Production of pectinolytic enzyme (polygalacturonase) was investigated.

MATERIALS AND METHODS

Source and Sampling

Soil as sources of yeasts used for this experiment was obtained from Bogor and Central Java. The sample of soil was collected from fruit plantation (mango, papaya, and banana), soil, and soil from garden.

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Isolation of Yeasts

Yeasts were isolated from soil samples by using a medium containing (per litre) 5g pectin, 4g $(NH_4)_2SO_4$, 2g KH_2PO_4, 1g K_2HPO_4, 0.4g $MgSO_4.7H_2O$, 3mg Fe citrate, 0.5mg $MnSO_4.4H_2O$, 0.05mg $CuSO_4.5H_2O$, 0.4mg $ZnSO_4.7H_2O$, 0.2g yeast extract, pH of 5.0 (isolated medium). Enrichment culture were carried out for the isolation of pectin assimilating yeasts. Large test tubes containing 15ml of enriched medium were inoculated aseptically with approximately one gram of sample soil. This culture was shaked and incubated aerobically at 28°C for four days, then transferred to another test tube containing 1ml enriched medium.

Afterward, enriched cultures were streaked on plate of isolation medium containing 2% agar, and then incubated at 28°C for three days. Isolated yeast strains were maintained using YM medium containing 2% agar. Pure culture of the yeasts were obtained by means of streaking techniques. The culture of each soil sample was repeated three times.

Identification of Yeasts

The strains used for this study are listed in Table 1. They were isolated from different sources such as soil. The first identification test (DNase test) comparised Urea hydrolysis (Urea test), Extracellular DNase (DNase test), Diazonium Blue B reaction (DBB test), and Yeast Fermentation of D-Glucose (YFG test).

The yeasts isolated were tested for their characteristics of vegetative reproduction, sexual characteristics by the methods described by Kreger-Van Rij (1984), Barnett *et.al.* (2000), and Guilliermond and Tanner (2006).

Investigation of Pectinolytic Enzyme

One strain were selected from each group (strain no. 111, 123, and 211), and determined for production of pectinolytic enzyme (polygalacturonase). The test was based on the method previously described by Endo (1961), Kozaki *et al.* (1980) and Kaur *et al.* (2004), with some modification. Media containing 2% pectin (sigma), 0.6% peptone, 0.2% yeast extract were used for the production of the pectinolytic enzyme. One unit of Polygalacturonase (PGase) is defined as the amount of enzyme that liberates 1 µmol of galacturonic acid min^{-1} ml^{-1} under the assay conditions.

RESULTS AND DISCUSSION

Nine yeast strains have been isolated from soil. Soil sampled in fruit plantation, and soil from garden in west Java and Central Java. Most isolated yeasts were obtained from medium containing pectin with enrichment culture. The source and location of strains were listed in Table 1.

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RESULTS AND DISCUSSION

Nine yeast strains have been isolated from soil. Soil sampled in fruit plantation, and soil from garden in west Java and Central Java. Most isolated yeasts were obtained from medium containing pectin with enrichment culture. The source and location of strains were listed in Table 1.

The first identification test comprised Urease test, DNase test (Sen and Komagata, 1979), DBB test, and YFG test. The result indicated that there are some yeasts that incapable of fermenting D-glucose (Table 2).

Test of morphological characteristics by using the method previously described by Kreger-Van Rij (1984) indicate that there were differences in colour of colony and cell shape, and the preserce of pseudo or true mycelium also presence. These characteristics could be used in a yeast grouping (Table 2 and Table 3)

Table 1. Source of isolates

No.	Strain No.	Source
1	111	Soil from mango field, Bogor-West Java
2	121	Soil from papaya field, Bogor-West Java
3	122	Soil from papaya field, Bogor-West Java
4	123	Soil from papaya field, Bogor-West Java
5	131	Soil from banana field, Bogor-West Java
6	211	Soil from garden, Wleri-Central Java
7	212	Soil from garden, Wleri-Central Java
8	213	Soil from garden, Wleri-Central Java
9	221	Soil from garbage, Wleri-Central Java

Table 2. Characteristic of isolated strains

No	Strain No.	Urease test.	DNase test	DBB test	YFG test
1	111	-	-	-	+
2	221	-	-	-	+
3	131	-	-	-	+
4	121	+	+	+	-
5	122	+	+	+	-
6	123	+	+	+	-
7	213	+	+	+	-
8	211	-	-	-	+
9	212	-	-	-	+

Table 3. Morphological characteristic of strains

No	Strain No.	Color of Colony	Cell size (µm)	Cell Shape	Type of Growth	Veg. Repr.	Pseudo or true mycel	Group
1	111	white	(2.5-7.0) x (4.0-10.5)	oval	pellicle	m.b.	+	I
2	221	white	(4.0-6.0) x (4.0-9.0)	oval	pellicle	m.b.	+	I
3	131	white	(3.5-5.0) x (5.0-10.0)	oval	ring	m.b.	+	I
4	121	brownish red	(2.5-5.5) x (3.0-8.0)	oval	ring	m.b.	-	II
5	122	brownish red	(2.5-6.0) x (3.0-9.5)	oval	islets	m.b.	-	II
6	123	brownish red	(2.3-5.8) x (2.8-7.8)	oval	ring	m.b.	-	II
7	213	brownish red	(2.5-6.0) x (2.8-7.5)	oval	ring	m.b.	-	II
8	211	white to cream	(2.5-6.5) x (3.5-10.0)	oval	wrinkle	m.b.	+	III
9	212	white to cream	(3.5-6.2) x (3.0-9.5)	oval	wrinkle	m.b.	+	III

m.b. : multilateral budding

Based on their physiological and biochemical characteristics identified with involved the method previously describe by Barnett *et al.* (2000), Guilliermond and Tanner (2006) suggested that the isolated strains could be devided into three groups (Table 4).

From three groups, one strain representing the groups were selected to test whether they have pectinolytic enzyme or not. The presence of pectinolytic enzyme such as polygalacturonase was investigated. All of strains produced polygalacturonase of 1.7 U. ml^{-1} (group I), 1.7 $U.ml^{-1}$ (group II), 1.0 $U.ml^{-1}$ (group III), respectively.

Table 4. Physiological and biochemical characteristic of isolated strains in group I, group II, and group III

Characteristics	Group I Strain No.			Group II Strain No.				Group III Strain No.	
	111	221	131	121	122	123	213	211	212
Fermentation									
D-Glucose	+	+	+	-	-	-	-	+	+
D-Galactose	+	+	+	-	-	-	-	-	-
Maltose	+	+	+	-	-	-	-	-	-
Sucrose	+	+	+	-	-	-	-	+	+
α,α-Trehalose	+	+	+	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-
Cellobiose	-	-	-	-	-	-	-	vw	vw
Raffinose	-	-	-	-	-	-	-	+	+
Me-D-glucoside	-	-	-	-	-	-	-	-	-
Aerobic Utilization									
D-Glucose	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	-	-
L-Sorbose	+	+	+	w	w	w	w	-	-
D-Ribose	-	-	-	+	+	+	+	-	-
D-xylose	+	+	+	+	+	+	+	+	+
L-Arabinose	-	-	-	+	+	+	+	-	-
D-Arabinose	-	-	-	+	+	+	+	-	-
L-Rhamnose	vw	-	-	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	-	-
α,α-Trehalose	+	+	+	+	+	+	+	-	-
Me-D-glucoside	+	+	+	+	+	+	+	-	-
Cellobiose	+	+	+	+	+	+	+	+	+
Salicin	vw	-	-	+	+	+	+	+	+
Melibiose	-	-	-	+	+	+	+	-	-
Lactose	+	+	+	+	+	+	+	-	-
Raffinose	-	-	-	-	-	-	-	+	+
Melezitose	+	+	+	+	+	+	+	-	-
Inulin	-	-	-	vw	vw	vw	vw	-?	-?
Starch	-	-	-	w	+	+	+	-	-
Erythritol	-	-	-	+	+	+	+	-	-
Ribitol	+	+	+	+	+	+	+	-	-
D-Mannitol	+	+	+	+	+	+	+	+	+
Ethanol	+	+	+	vw	vw	vw	vw	+	+
Galactitol	vw	-	-	+	+	+	+	-	-
Xylitol	-	-	-	+	+	+	+	+	+
Inositol	-	-	-	+	+	+	+	-	-
D-Gluconate	+	+	+	+	+	+	+	+	+
Succinate	+	+	+	+	+	+	+	+	+
DL-Lactate	w	w	vw	-	-	-	-	+	+
Citrate	+	+	+	-	-	-	-	+	+
D-Glucuronate	-	-	-	+	+	+	+	-	-

Table 4. Physiological and biochemical characteristic of isolated strains in group I, group II, and group III (*continous*...)

Characteristics	Group I Strain No.			Group II Strain No.				Group III Strain No.	
	111	221	131	121	122	123	213	211	212
Amonium	+	+	+	+	+	+	+	+	+
Nitrite	-	-	-	-	-	-	-	+	+
Nitrate	-	-	-	-	-	-	-	+	+
Ethylamine	+	+	+	+	+	+	+	+	+
Lysine	+	+	+	+	+	+	+	+	+
Glucosamine	w	+	+	+	+	+	+	-	-
Cadaverine	+	+	+	+	+	+	+	+	+
50% D-Glucose	-	vw	w	-	-	-	-	-	-
60% D-Glucose	-	-	-	-	-	-	-	-	-
1% acetic acid	-	-	-	-	-	-	-	-	-
0.01% cycloheximide	-	+	+	-	-	-	-	-	-
0.1% cycloheximide	-	-	-	-	-	-	-	-	-
w/o vitamins	+	+	+	+	+	+	+	+	+
w/o thiamin	+	+	+	+	+	+	+	+	+
At 15°C	+	+	+	+	+	+	+	+	+
20°C	+	+	+	+	+	+	+	+	+
25°C	+	+	+	+	+	+	+	+	+
30°C	+	+	+	+	+	+	+	+	+
37°C	+	+	+	-	-	-	-	-	-
40°C	+	+	+	-	-	-	-	-	-
45°C	w	+	+	-	-	-	-	-	-
50°C	-	-	-	-	-	-	-	-	-
In 10% NaCl	+	+	+	vw	vw	vw	vw	-	-
15% NaCl	w	vw	vw	-	-	-	-	-	-
20% NaCl	-	-	-	-	-	-	-	-	-
25% NaCl	-	-	-	-	-	-	-	-	-
Acid production	w	w	w	-	-	vw	-	w	w
Starch formation	-	-	-	-	-	-	-	-	-

w : weak

vw : very weak

Group I consisted of 3 strains, group II had 4 strains, and group III had 2 strains (Table 3 and Table 4). Each group is discussed separately as follows:

Group I

All strains in this group had negative respons to urease test, DBB test, and extracelluler DNase test (Table 2). The yeast culture growth liquid medium produced pellicle, except strain no. 131 that produced a ring on the surface of the medium. The strains produced pseudomycelia and were likely to form septate hyphae. Comparing the observed yeast characteristics with that described by Banett *et al.* (2000), group I was closely related to *Candida tropicalis*. The yeasts formed a septate hyphae, had a similar fermentation pattern and grew well at a temperature of 40 °C.

Physiological and biochemical characteristics of group I were similar to those of *C. tropicalis*. However, the growth of strains in group I in lactose medium were positive, in starch were negative, in glucosamine (N) were positive or weak, and in 50% D-glucose were negative or very weak or weak. There were a small differences among the strain in group I in assimilating L-rhamnose, salicin, and galactitol (Table 4). The strain no. 111 exhibited a very weak response, whereas the other strains showed a negative response. The strains in group I were interesting in the growth at a temperature of 40 °C. Their adaptability to high temperature seemed to be related to

sampling site, as the sources were obtained from tropical region. *C. tropicalis* as reported by Barnett *et al.* (2000), was found in rotten fruit, soil, and soil from fruit plantation, where all strains in group I were selected.

Group II

The strains in this group showed a negative response on fermentation test and a similar result in aerobic utilization test. There was an exception result found in starch assimilation test in no. 121, which had a weak response. Strain formed a multilateral budding and might have capsulated cells, the culture on solid media are generally mucous. Color of the colony was brownish-red. There was no fermentation, and no utlization of nitrate and nitrite. Positive reaction were found for urease, DBB reaction, and DNase tests (Table 2).

Comparing result of morphological and physiological characteristization with those described by Kreger-Van Rij (1984) and Barnett *et al* (2000), the strains in group II were similar with genus *Rhodotorula*. The spesies in genus *Rhodotorula Harrison* had characteristics as follow : multilateral budding, the cultures were red or yellow due to carotenoid pigments, the cultures were often mucous, had no fermentation, nitrat was assimilated or not, inositol was not assimilated, starch-like compounds were not produced, urease test was positive. Strain in group II had speciality over the other groups as they assimilated lactose and inositol as carbon sources. However, some species in genus *Rhodotorula* assimilated lactose, but did not assimilated inositol.

Group III

Isolated strains included into group III had different characteristic when they were compared with other groups, especially in nitrate and nitrite assimilation showed by a positive reaction. The strains in this group had a similar morphological and physiological characteristics. Inulin test failed to demonstrate wether they assimilated the inulin or not. Kreger-Van Rij (1984) describe that genus *Hansenula* H. *et* P. Sydow had characteristics as follow : multilateral budding; pseudo or true-mycelium might be present; ascospore hat-saturn-shaped or hemispherical were generally liberated; fermentation was present or absent; and assimilated nitrate. These description were very close to the morphological and psychological characteristics of isolated strain in group III, therefore the could be classified as genus *Hansenula*.

The isolated strains had also evanescent and persistent asci, containing 1 to 4 saturn-shape ascospore. This result showed that the strains were closely related to species *Williopsis saturnus* (Barnett *et.al.*, 2000) which was the synonyms of *Hansenula saturnus* var. *saturnus* and *Hansenula saturnus* var. *subsufficiens*. Characteristics of isolated strains in group III differed slightly from species *Williopsis saturnus*. The strains in group III fermented cellobiose but the response was very weak (Table 4). The other characteristics were similar.

Pectinolytic Enzyme

The enzymes that hydrolaise pectic substances are broadly known as pectinolytic enzymes or pectinases, which include polygalacturonase (PGase), pectin esterase, pectin lyase, and pectate lyase on the basis of their mode of action (Alkorta *et al.* 1998). Pectinase have widespread application in food and textile industries (Henriksson *et al.* 1999).

The activity of polygalacturonase enzyme was observed in group I, II, III. These groups produced polygalacturonase, if pectin was used as a carbon source for enzyme production. Strains tested exhibited low activity (1.0-1.7 U.ml^{-1}). Serrat *et al.* (2004) reported the activity of poligalacturonase of 4.19 U.ml^{-1} in YNB (Difco) – glucose medium from the *Kluyveromyces marxianus* CCEBI 2011 yeast strain. However, polygalacturonase production by the thermophilic mould *Sporotrichum thermophile* Apinis was high (about 30 U.ml^{-1}) in pectin 2% (Kaur *et al.*, 2004).

CONCLUSIONS

The strains isolated were taxonomically separated into 3 groups. Group I contains 3 strains, and this group is closely related to *Candida tropicalis*. Group II contains 4 strains, and this group is included in genus *Rhodotorula*. Group III contains 2 strains, and this group is closely related to *Williopsis saturnus*, which is a synonym of *Hansenula saturnus*.

Pectinolytic enzymes (Polygalacturonase) were produced by all of the tested strains. Polygalacturonase was produced as high as 1.7 U.ml^{-1} by strain no. 111 of group I, 1.7 U.ml^{-1} by strains no. 123 of group II, and 1.0 U.ml^{-1} by strain no. 211 of group III, respectively.

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