CHARACTERISTICS OF THE PEAT SOIL *Bacilllus* sp.

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ABSTRACT

Twenty two strains of Bacillus *species have been isolated from the peat soil of Tanjung Puting, Central Kalimantan. All strains devided into seventeen groups which characterized using morphology and biochemical tests. All strains have been tested for lipase, amylase, protease, cellulase, and phosphatase activity. Thirteen* Bacillus *isolates tested gave positif reaction for lipase activity.* Bacillus *AR 018 was the best isolate for amylase activity,* Bacillus *AR 001 and* Bacillus *AR 002 for protease activity,* Bacillus *AR 009 for cellulase activity. Only one isolate shows positive reaction for phosphatase activity, notably* Bacillus *AR 007.*

Key words: Bacillus*, peat soil, lipase, amylase, protease, cellulase, phosphatase*

INTRODUCTION

Peat swamp is the one of the unique ecosystems in the tropical rain forest, especially in Indonesia. Peat is the term for partially decomposed organic matter that has accumulated in a moist environment. This decay of organic material into peat, called the humification process, is brought about partly by microbes. The soil acidity is very acid with pH range from 3.8 to 5.0 and organic matter composition greater than 65% (Brady, 1990).

In various ecosystems including peat soil ecosystem, microbes is the most active and dynamic fraction of the living organic pool. Microbial activity in soils play a fundamental role in determining the availability of nutrients for plant growth, and microbial transformations often supply a substantial quantity of plant nutrients. The soil microbes is instrumental in the degradation and synthesis of organic compound. Bacteria is the most dominant microbial in the soil besides fungi. Vast number of bacteria which dominated in the soil is genus *Bacillus*. The *Bacillus* community have a wide range of distribution in environment.

However, verification on the role of *Bacillus* species in the peat swamp soil has not been reported, therefore isolation and characterization of this genus might provide better information on their ecological role, and finally the possible use of its for biotechnology application.

MATERIALS AND METHODS

Culture Media

Nutrient agar medium used for isolation and maintenance of the *Bacillus* contained (gl^{-1}): bacto peptone 5; beef extract 3; agar 15. The final pH of the freshly prepared medium was 6.8. Sterilized by autoclaved.

Isolation of Bacteria

Isolation of *Bacillus* sp. prefer to Nakayama (1981) method. Heating the soil sample at 80° C in 5 minutes. Spreading 0.1 g soil sample on the nutrient agar medium.

Gram test

Test for Gram reaction reference by Busse (1999) method. Scrape the growth from a slant or plate, and suspend it in a drop of KOH 3%.

Endospores

The description of endospores shape and position was based on the Schaeffer and Fulton technique (Levine, 1954). Flood the dried smear with malachite green and heat to steaming within one-half minute. Wash off excess stain for about one-half minute. Apply safranin solution for one-half minute. Wash in water and dry.

Catalase

Test for catalase were made as described by Smibert and Krieg (1994). Scrape the growth from a slant or plate with a nonmetallic instrument, and suspend it in a drop of 3% hydrogen peroxide on a slide.

Gelatin Hydrolysis

Streak the plates of agar medium supplemented with 0.4% gelatin. Incubate them at the optimum temperature for the organism. Flood the plates with gelatin-precipitating reagent (15% $HgCl_2$ in 20% (v/v) concentrated HCl) (Smibert and Krieg, 1994).

Motility

Test for motility were made as described by Levine (1954). The medium contained (gl^{-1}): peptone 5; beef

extract 5; agar 15. Inoculation of bacteria with a stright needle in the center of the tube about ½ inch long. Incubate at the body temperature for 24 hour.

Carbohydrate Catabolize

Hugh and Leifson's medium with 1% sugar (gl^{-1}): peptone (pancreatic digest of casein) 2; NaCl 5; K_2HPO_4 0.3; agar 3. Combine ingredients and adjust the pH 7.1. Incubate at 28° C for up to 14 days (Smibert and Krieg, 1994).

Nitrite Production

Inoculate a suitable broth medium supplemented with 0.01 to 0.10% $NaNO_3$ or KNO_3 and 0.17% agar. Gently mix the inoculum with the medium to distributed throughout the tube. Add 2 ml of nitrite test reagents (Smibert and Krieg, 1994).

Casein Hydrolysis

Combine sterile (autoclaved) skim milk at 50° C with an equal volume of double strength nutrient agar or other carbohydrate free agar medium at 50° C to 55° C. Incubate streaked plates for up to 14 days and look for clear zones surrounding the growth. Confirm by floading the plates with 10% HCl (Smibert and Krieg, 1994).

Acid from Dextrose

Inoculate of fresh prepared dextrose peptone broth with organism. Incubate at 37° C for 24 to 48 hours. Medium composition (gl^{-1}): peptone 5; dextrose 5 (Levine, 1954).

Lipase Activity

Streak the plates of egg yolk agar which contained: peptone 20 g; Na_2HPO_4 2.5 g; NaCl 1 g; $MgSO_4.7H_2O$ 0.5% (w/v) solution 0.1 ml; glucose 1 g; agar 12.5 g; distilled water 500 ml. Incubate them at the optimum temperature for the organism (Smibert and Krieg, 1994).

Amylase Activity

Test for amylolytic bacterial were made as describes by Smibert and Krieg (1994). The medium contained (gl^{-1}): peptone 5; beef extract 3; NaCl 5; starch 2; agar 15. Incubate at the room temperature.

Protease Activity

Test for proteolytic activity were made as described by Rodina (1972). The medium of frazier gelatin contained: NaCl 3 g; K_2HPO_4 1.5 g; KH_2PO_4 0.5 g; gelatin 4 g; dextrose 0.05 g; peptone 0.1 g; beef extract 5 ml; agar 15 g; distilled water 1000 ml. After incubation, flood the plate with the following solution: $HgCl_2$ 15 g; HCl 20 ml; distilled water 100 ml.

Cellulase Activity

Inoculate the organism on the cytophage agar medium which contained (gl^{-1}): $(NH_4)_2SO_4$ 1; $MgSO_4.7H_2O$ 0.1; yeast extract 1; $FeCl_3$ 0.01; glucose 1; carboxymethylcellulose 1%. Agar plates were incubated for 28° C for 3 to 7 days. After suitable incubation time, congo red was used as an indicator for detection and enumeration of cellulytic colonies, as described by Mullings and Parish (1984).

Phosphatase Activity

Test for phosphatase activity were made as described by Gaur (1981). The medium contained (gl^{-1}): $Ca_3(PO_4)_2$ 5; glucose 10; NaCl 0.2; KCl 0.2; $MgSO_4.7H_2O$ 0.1; $MnSO_4.H_2O$ 0.0025; $FeSO_4.7H_2O$ 0.0025; yeast extract 0.5; agar 15. Agar plates were incubated at 28° C.

RESULTS

Phenotypic Characterization

Phenotypic characterization of the *Bacillus* isolates studied are shown in Table 1 and Table 2 respectively. Based on the morphological and position of endospores, and biochemical test, 22 *Bacillus* isolates divided into 13 groups (Table 3).

Table 1. Endospores shape and position in sporangium of *Bacillus* which isolation from the peat soil, Tanjung Puting, Central Kalimantan

Bacillus isolates	Endospore shape	Endospore position
AR 001	Oval	Sub terminal
AR 002	Ellips	Central
AR 003	Ellips	Central
AR 004	Ellips	Sub terminal
AR 005	Ellips	Sub terminal
AR 006	Ellips	Sub terminal
AR 007	Ellips	Sub terminal
AR 008	Oval	Sub terminal
AR 009	Ellips	Sub terminal
AR 010	Oval	Sub terminal
AR 011	Ellips	Sub terminal
AR 012	Ellips	Sub terminal
AR 013	Ellips	Sub terminal
AR 014	Oval	Sub terminal
AR 015	Ellips	Sub terminal
AR 016	Oval	Sub terminal
AR 017	Spherical	Terminal (swelling)
AR 018	Oval	Sub terminal
AR 019	Oval	Sub terminal
AR 020	Oval	Sub terminal
AR 021	Ellips	Sub terminal
AR 022	Ellips	Central

Table 2. Phenotypic characteristics of *Bacillus* which isolation from the peat soil, Tanjung Puting, Central Kalimantan

Bacillus isolates	Gram test	Endospore examination	Catalase test	Motility	Carbohydrate catabolize	Acid from dextrose	Gelatin liquefaction	Nitrate test	Casein hydrolysis
AR 001	+	+	+	+	+	-	+	+	+
AR 002	+	+	+	+	+	+	+	+	+
AR 003	+	+	+	+	+	+	-	+	+
AR 004	+	+	+	+	+	+	-	+	+
AR 005	+	+	+	+	+	+	-	+	+
AR 006	+	+	+	+	-	+	+	+	+
AR 007	+	+	+	-	+	+	-	+	-
AR 008	+	+	+	+	+	-	-	+	+
AR 009	+	+	+	+	-	+	+	+	+
AR 010	+	+	+	-	-	+	+	+	+
AR 011	+	+	+	-	-	+	-	-	+
AR 012	+	+	+	-	-	+	+	+	+
AR 013	+	+	+	-	-	+	+	+	+
AR 014	+	+	+	+	+	+	-	+	+
AR 015	+	+	+	-	-	+	+	-	+
AR 016	+	+	+	+	+	-	+	+	+
AR 017	+	+	+	+	-	-	+	-	-
AR 018	+	+	+	+	+	+	+	+	+
AR 019	+	+	+	+	+	+	+	+	+
AR 020	+	+	+	+	-	+	-	-	+
AR 021	+	+	+	+	+	-	+	+	+
AR 022	+	+	+	+	+	-	+	+	+

Group I contained two isolates (AR 001 and AR 016). All isolates in this group did not have an ability to produce acid from dextrose, but shows a positive reaction on the gelatin liquefaction, nitrate test, and casein hydrolysis test.

Group II only contained one isolate (AR 021). This group shows the same reaction within biochemistry test with group I. Morphology of endospore is the things to distinguish this group with group I.

Group III contained one isolate (AR 022). Morphology and position of endospore of this group is different with group I and group II respectively. Conversely, this group has the same reaction within biochemical test with two groups above.

Group IV only contained one isolate (AR 002). This group shows a positive reaction in the all of biochemical test.

Two isolates were belong to group V (AR 018 and AR 019). Morphology and endospore position is the factor to distinguish this group with group IV above.

Group VI only contained one isolate (AR 003) the isolate was negative for gelatin liquefaction.

Two isolates were belong to group VII (AR 004 and AR 005). These isolates have the same biochemical characteristics with group VI. The position of endospore of this group is sub terminal.

Group VIII only contained one isolate (AR 014). The isolate was positive for mostly biochemical test except gelatin liquefaction. The endospore form was oval.

Group IX contained two isolates (AR 006 and AR 009). They gave negative reaction for carbohydrate catabolize.

Group X only contained one isolate (AR 007). This group was non motile and gave negative reaction for gelatin liquefaction and casein hydrolysis.

Group XI only contained one isolate (AR 008). This group did not produce acid from dextrose and shows negative reaction for gelatin liquefaction.

One isolate belong to group XII (AR 010). This group gave negative reaction for carbohydrate catabolize and non motile.

Group XIII contained two isolates (AR 012 and AR 013). The member of this group shows the same biochemical reaction with group XII. Conversely, the form of endospore was different. This group have an oval endospore.

Group XIV only contained one isolate (AR 011). This group has ability to produce acid from dextrose and gave positive reaction for casein hydrolysis. For carbohydrate catabolize, gelatin liquefaction, and nitrate test the isolate gave negative reaction.

Group XV contained one isolate (AR 015). It also non motile, gave negative reaction for carbohydrate catabolize and nitrate test.

Only one isolate belonged to group XVI (AR 017). Positive reaction were observed in gelatin liquefaction. Endospore form was swelling and terminal in the position.

Group XVII contained one isolate (AR 020). This group shows a positive reaction for casein hydrolysis and was able to produce acid from dextrose. Negative reaction showed on the carbohydrate catabolize, gelatin liquefaction, and nitrate test.

Table 3. Grouping of *Bacillus* isolates based on phenotypic characterization

Groups	*Bacillus* isolates
I	AR 001, AR 016
II	AR 021
III	AR 022
IV	AR 002
V	AR 018, AR 019
VI	AR 003
VII	AR 004, AR 005
VIII	AR 014
IX	AR 006, AR 009
X	AR 007
XI	AR 008
XII	AR 010
XIII	AR 012, AR 013
XIV	AR 011
XV	AR 015
XVI	AR 017
XVII	AR 020

Vast number of *Bacillus* species tested was able to produce lipase, amylase, protease, and cellulase. It was showed in the Table 4, approximately 13 isolates of *Bacillus* species tested have an ability to produce lipase enzymes.

In this preliminary studies showed that 18 isolates of *Bacillus* tested have a positive reaction on the starch agar plates. *Bacillus* AR 018 has a greatest amylase index in this study. *Bacillus* AR 001 and AR 002 were the best *Bacillus* isolates which capable to degrade gelatin as a protease study. Table 4 presents data on the capability of some species of *Bacillus* to decompose cellulose. From the table shows that *Bacillus* AR 009 was the highest cellulolytic bacteria which degrade cellulose on the cytophaga agar medium. The relative activity of this isolate is 9.00. Conversely, *Bacillus* AR 020 has a smallest relative activity on the cytophaga agar medium, notably 2.40. Some isolates like *Bacillus* AR 007, AR 010, AR 015, AR 017, and AR 019 did not degrade cellulose.

Only *Bacillus* AR 007 was able to degrade $Ca_3(PO_4)_2$, although the clear zone surrounding the colony was small. From this study shows that phosphate solubilizing influenced by soil type particularly of certain soil. Beside pH of soil, several factors are responsible to affect phosphorous dissolution such as macro element and decomposition stage of organic substrates (Brady, 1990).

Table 4. Enzymes activity of *Bacillus* which isolation from the peat soil, Tanjung Puting, Central Kalimantan

Bacillus isolates	Lipase test[a]	Amylase index[b]	Protease index[b]	Cellulase index[b]	Phosphatase index[b]
AR 001	–	1.60	4.33	5.00	–
AR 002	+	1.25	4.20	4.60	–
AR 003	–	1.34	1.38	4.75	–
AR 004	–	1.39	1.24	5.67	–
AR 005	+	–	1.18	4.40	–
AR 006	+	1.67	1.53	2.50	–
AR 007	+	–	–	–	1.33
AR 008	+	1.09	2.63	6.00	–
AR 009	–	1.62	1.69	9.00	–
AR 010	+	1.50	2.40	–	–
AR 011	+	–	1.15	7.50	–
AR 012	+	1.60	1.38	2.50	–
AR 013	+	2.20	1.58	3.00	–
AR 014	–	2.25	1.06	3.50	–
AR 015	+	2.25	3.58	–	–
AR 016	–	1.42	1.33	7.33	–
AR 017	+	–	–	–	–
AR 018	–	3.17	1.29	4.67	–
AR 019	+	1.28	2.17	–	–
AR 020	+	1.71	1.32	2.40	–
AR 021	–	2.33	1.15	4.33	–
AR 022	–	2.20	1.29	4.67	–

[a] a positive test is indicated by an oily, iridescent sheen or pearly layer over the colony and on the surface of the surrounding agar

[b] values of clear zone diameter/ colony diameter (milimetres)

DISCUSSION

Lipase (triacylglyceral acylhydrolases) are produced by various microorganisms either alone or together with esterase (Kouker and Jaeger, 1986). Main roles of lipase to degrade lipid mainly phospholipid into lipid acid. Generally, *Bacillus* species have ability to produce this enzymes. Examination of *Bacillus* species on egg yolk agar plates identified these strains as lipase producers. A direct quantitation of lipase is difficult, presumably because in the amounts of lipase molecules released by a single colony. A positive test is indicated by an oily, iridescent sheen or pearly layer over the colony and on the surface of the surrounding agar.

Generally, amylase produced by microorganisms used to degrade starch or amylum into small compounds such as maltose, maltotriose, and glucose. Further microorganisms utilize these compounds as a source energy for its growth. The composition and concentration of media greatly affect the growth and production of extracellular amylase in bacterial. The synthesis of amylase was good when the bacteria was grown on starch or other polysaccharides (Privastava and Baruah, 1986).

Vast number of *Bacillus* species have a capability to produce protease enzyme. Microbial proteases degrade protein to peptides and amino acids. Either these products are utilized directly by the *Bacillus*, or the amino acids are deaminated, giving rise to short-chain acids, carbondioxide, and ammonia. *Bacillus* have been reported to be primarily responsible for the degradation of plant protein. The various responses of different strains within the same species growing on the same substrate are perhaps not so result of variable possession of structure genes, the complexity of the response of certain isolate to substrate suggests that other factors are involved.

The species of bacteria, particularly *Bacillus* capable of cellulase decomposition are very numerous. *Bacillus* species having adapted themselves to different ecological conditions. Therefore, it may be detected in a wide range of soils. Cellulolytic activity of bacteria caused the presence of endo-â-1.4-glucanase enzyme. This enzyme have been broke the â-1.4-glicoside chain on the cellulose fiber.

Most of soil consist of organic and inorganic phosphorous. Phosphorous is essential element required by microorganisms for nucleotide synthesis and for plant photosynthesis (Tisdale *et al*., 1985). Out of 22 *Bacillus* species tested, only one isolate degrade $Ca_3(PO_4)_2$. This phenomena suggests that on the acid soil, the number of phosphatase *Bacillus* was low. From this study shows that phosphate solubilizing influenced by soil type particularly of certain soil. Beside pH of soil, several factors are responsible to affect phosphorous dissolution such as macro element and decomposition stage of organic substrates (Brady, 1990).

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