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Potency of phosphate solubilizing mold from rhizosphere soil in Mangrove Center Tuban, Indonesia

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ABSTRACT

This research aims to isolate phosphate solubilizing mold from the rhizosphere soil of Mangrove Center Tuban, East Java, Indonesia which can be used in biofertilizer. All isolates were described based on macroscopic and microscopic morphological characteristics. The phosphate solubilizing activity was measured by growing the isolates on Pikovskaya medium and measuring the phosphate solubilization index after 3-7 days incubation. The results show that from 60 obtained isolates, 33 mold isolates showed phosphate solubilizing activity. Mold isolates which showed the highest phosphate solubilizing activity index are isolates K.32, K.43, and K.58 with phosphate solubilization index 1.58, 1.66, and 1. 23, respectively. mold isolates of K.32 and K.58 belong to genera of *Aspergillus*, while K.43 belongs to the *Penicillium* genera.

Key words : *Phosphate solubilizing mold, Phosphate solubilization index, Mangrove Center Tuban*

Introduction

Indonesia is an archipelagic country that has an abundance of biodiversity and vast areas of mangrove forests. One of the coastal cities in the East Coast region of East Java, often called as East Java Pantura (Pantai Utara: North Coast) area, is Tuban Regency which has ± 65 km long beach (Ika, 2016). Mangrove Center Tuban (MCT) is one of the less explored area, especially for microbiology-related study. Microbes are microscopic microorganisms including bacteria, fungi, and *actinomycetes*, that contribute to the process of nutrient cycling in the productivity of mangrove ecosystems (Turjaman, 2017).

One of the critical main habitat of microbes is soil (Rodesia *et al.*, 2013). One critical element in the soil for plant growth is phosphate which available in both, organic and anorganic forms (Putri, 2014). The availability of phosphate in the soil is abundant, but most of them (95-99%) cannot be utilized by plants, due to their insolubility. Phosphate solubilizer microorganisms are able to release phospate through the secretion organic acids (Nasution, 2014). Some fungi are used as phosphorus solubilizing agents, especially from the genera of *Aspergillus, Penicillium, Mucor, Fusarium,* and *Candida* (Rai, 2006). This research aims to isolate molds with Phospate solubilizing potential from the rhizosphere of Mangrove Center Tuban for biofertilizer candidate.

Materials and Methods

The soil sampling was carried out at 2 locations. 10 sampling points. Location 1 8 sampling points is a former pond location with 10 points, and location 2

8 sampling point is nearby the river mouth with 8 points. Soil sampling was taken out around the rhizosphere of Mangrove Center Tuban soil using purposive sampling method with the soil samples were taken up with the depth of 0-15 cm (Martina *et al.*, 2013) using soil borer (Wahab *et al.*, 2015). Soil sample were taken as much as ± 250 g at each point and homogenized into sterile plastic bag. Then, it was stored to ice boxes, after that brought to the Laboratory, and stored at 4°C. Ten grams of the soil sample was suspended with 90 mL physiological saline solution (0.85% NaCl) in Erlenmeyer and homogenized (Martina *et al.*, 2013) using vortex for ± 15 minutes. Furthermore, the suspension was allowed to stand for ± 10 minutes, so that 1 mL could be taken from it. Next, a gradual dilution was carried out from 10^1-10^3 dilution, which was taken by 1 mL, and put into a Petri dish to be grown by pour plate method on Potato Dextrose Agar (PDA) medium (Rosita *et al.*, 2014). The culture was incubated at room temperature 25-27°C for 3-7 days.

Each different colony that grew was purified and grown on new PDA medium. The isolates was incubated at room temperature (25-27 °C) for 3-7 days. Pure isolates were stored at 4 °C until they were used. Identification of mold was done by 2 observations, macroscopic and microscopic characters. Macroscopic observations were done according to Ilyas (2007). Microscopic observation was using slide culture method (Hastuti, 2014). Molds Identification based on Molds identification book (Gandjar *et al.*, 1999; Landecker, 1996). Pure isolates were grown on Pikovskaya medium using spotted method (Zulaika *et al.*, 2015) at room temperature for 3-7 days. The clear zone formed around the colony is an indicator of the mold's ability to dissolve phosphate. The ratio of clear zone diameter to colony diameter was used to see and know the qualitative ability of isolates to dissolve phosphate (Martina *et al.*, 2013).

Results and Discussion

Based on environmental research data at the location of soil sampling, the soil conditions at Mangrove Center Tuban support the environmental conditions for mold to growth. In location 1 (former pond) there are many plant litters in the form of falling leaves, stems, or twigs with plant vegetation in the form of *Rhizopora apiculata* and has loose, textured soil. The soil pH 4-6.4, temperatures range 27-28 °C, soil moisture was 7-8%, 79-82% of air humidity, and 2-15% of water salinity. While the location 2 (near the estuary) has a slightly muddy textured soil with a brownish color that is in direct contact with tides, plant vegetation in Location 2 is *Rhizopora apiculata*. The soil conditions have pH between 4.8-6.3, soil temperature 28-29 °C, 8% of soil moisture, 70-89% of air humidity, and 0°/$_{oo}$ of water salinity. According to Waluyo (2007) molds are able to grow at the optimum temperature, which is around 25-30 °C with an optimum pH of 5-7 under acidic conditions. In addition, a low salinity level will provide a good growth response for the plant and microbes (Hutahaean *et al.*, 2008).

Isolation from rhizosphere soil of Mangrove Cen-

Table 1. Phosphate Solubilizing Molds from Rhizosphere of Mangrove Center Tuban

No.	Isolate codes	Colony diameter (mm)	Clear zone diameter (mm)	Activity index
1.	K.3	20.75	29.75	0.43
2	K.4	7.80	8.30	0.06
3	K.6	18.50	29.25	0.58
4	K.7	18.35	25.55	0.39
5	K.8	19.55	27.55	0.41
6	K.9	9.40	17.40	0.85
7	K.10	14.65	15.40	0.05
8	K.11	22.40	31.85	0.42
9	K.12	80.60	81.85	0.02
10	K.13	22.35	33.20	0.49
11	K.14	20.25	29.10	0.44
12	K.15	20.05	23.55	0.17
13	K.16	29.60	35.70	0.21
14	K.17	21.40	27.30	0.28
15	K.18	18.85	26.85	0.42
16	K.19	27.90	31.15	0.12
17	K.20	11.10	12.90	0.16
18	K.22	6.60	7.15	0.08
19	K.23	45.75	49.55	0.08
20	K.24	26.30	36.50	0.39
21	K.32	22.00	56.70	1.58
22	K.35	28.40	34.10	0.20
23	K.39	34.80	43.50	0.25
24	K.41	35.75	39.80	0.11
25	K.42	16.70	21.60	0.29
26	K.43	9.65	25.65	1.66
27	K.44	39.50	63.6	0.61
28	K.46	11.10	21.70	0.95
29	K.52	81.10	81.50	0.01
30	K.54	82.30	82.80	0.01
31	K.55	24.35	35.40	0.45
32	K.58	19.65	43.75	1.23
33	K.59	18.65	34.70	0.86

ter Tuban managed to get 60 isolates (Table 1) including 33 isolates which are capable to produce phosphatase enzymes by forming a clear zone around the colonies on Pikovskaya medium, while 18 isolates grow with the same clear zone diameter with mold colonies. 9 isolates did not experience growth in Pikovskaya medium. Mold isolates that able to produce clear zones are caused by the hydrolysis process by the phosphatase enzyme. Pikovskaya media is used as a selective medium for phosphate solubilizer. This is caused by the composition of selective media can help inhibit the growth of undesirable microbes in accordance with their main objectives so that certain microbial groups have the potential to grow (Murtius *et al.*, 2017).

From 33 isolates, Three isolates with highest phosphate solubilization index have been identified macros and microscopically. K.32 isolate had an phosphate solubilization index of 1.58. It was grown on PDA and it had macroscopic characteristics such as irregular colony, granular texture, having a radial furrow and zone growth. Furthermore, K.32 did not have zonation and exudate drops. The surface color of the colony was light brown with white edges, while the reverse side was creamy.

K.32 isolate has hyphae with septa, with the structure of the cell wall, cell nucleus, and septum which can be observed. The conidial head is round and small, the conidiophores are smooth-walled, the vesicles are round, the phialides are formed in the metulae, and the conidia are round in size 3.5 µm (Figure 1). K.43 isolate has a phosphate solubilization index of 1.66 with macroscopic characteristics irregular shape, radial furrow, zone growth and exudate drops. But, it does not have zonation. The surface color of the colony is bluish green and velvety textured, while the radial are white in the edge and the color of the reversed side is yellow. K.43 isolate have hyphae with septa, conidiophores with long and stingy conidias, conidial head resembling a fork and hyaline, metulae measuring 8µm x 2µm which is somewhat cylindrical and smooth-walled, round conidia with 2.5 µm size (Figure 2).

K.58 isolate has a Isolate index of 1.23. K.58 isolate has macroscopic characteristics, rounded colony shape, granular colony texture, has radial furrow and zone growth but has no zonation and exudate drops. The surface side shows brownish with yellowish edges, while the color of the reverse side is creamy. Microscopic characteristics of K.58 were hyphae with septa, a small conidial head smooth-walled conidiophores, round vesicles, phialid

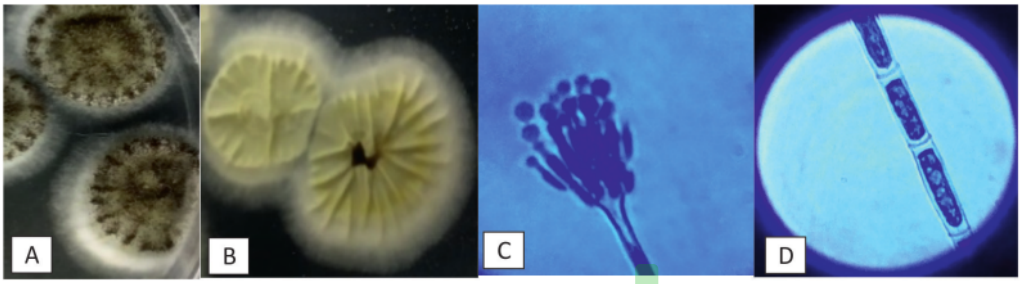

Fig. 1. Isolates K.32 macroscopically and microscopically (A: surface side of colony; B: reverse side of colony; C : conidiospores, D : conidiophore)

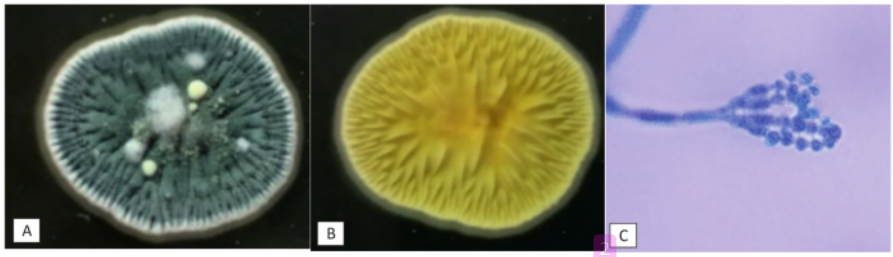

Fig. 2. Macroscopic and microscopic characters of K.43 isolate (A: surface side of colony; B: reverse side of colony; C: conidiospore)

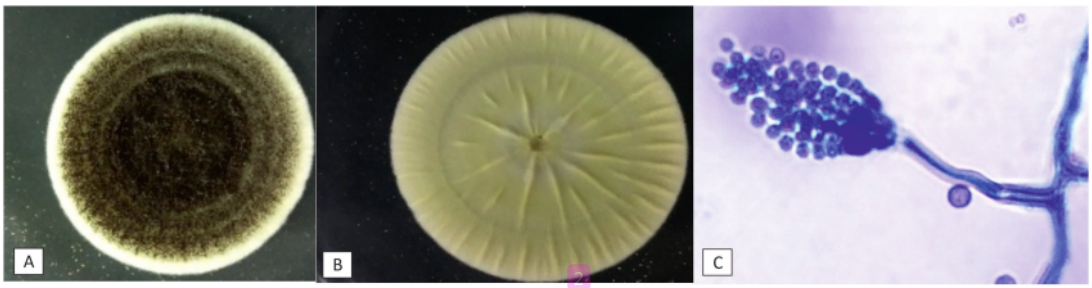

Fig. 3. Macroscopic and microscopic isolates K.58 (A: surface side of colony; B: reverse side of colony; C: Conidiospore with sected hyphae)

formed on hyaline-colored metulae, and round-shaped conidia with 5 µm in size (Figure 3).

Based on Gandjar (1999), isolates K.32 and K.58 were identified as genus *Aspergillus*, while isolate K.43 as genus *Penicillium*. This research obtained 60 isolates consist of the genera from *Aspergillus*, *Penicillium, Trichoderma*, and *Rhizopus*. According to Susanto *et al.,* (2013), mold is one of the microbial groups that has an important role in biofertilizer. Biofertilizer agents from mold such as *Penicillium*, *Fusarium*, and *Aspergillus* are able to dissolve phosphate bound to phosphate that can be utilized and available in the soil.

Conclusion

This research is succeeded in obtaining 60 isolates including 33 isolates that have the potential to dissolve phosphate from the rhizosphere soil of Mangrove Center Tuban, East Java. Three isolates with the highest phosphate solubilization index are isolate K.32 and K.58 which were identified as *Aspergillus sp*, while isolate K.43 as *Penicillium sp*.

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