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Potency of phosphate solubilizing Yeast from Mangrove Center in Jenu, Tuban, Indonesia

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ABSTRACT

The purpose of this research was to isolate potential phosphate solubilizing yeasts from rhizosphere soil in Mangrove Center Jenu Tuban, Indonesia. Soil samples were collected using purposive sampling technique. Yeast were isolated from the soil and purified using YMEA (Yeast Malt Extract Agar) medium. These isolates were inoculated in Pikovskaya Agar medium using spot inoculation method, and incubated for 3 days to screen for the phosphate solubilizing activity. The positive result was indicated by formation of clear zone (halo zone) surrounding growing colonies. Seventeen yeasts indicated the potency of phosphate solubilizer. Isolates with the highest potential were K14, K1, and K8 with *Phospate Solubilizing Indexes* (PSI) 2.58, 2.55, and 2.48, respectively. K14 was identified as the member of genus *Candida.*

Key words : *Phophate solubilizing index, Yeast, Mangrove center Jenu Tuban.*

Introduction

Chemical fertilizers have widely used by Indonesian farmers in agricultural activities. However, chemical fertilizers might have negative effects such as include waterway pollution, increased air pollution, soil acidification, and mineral depletion (Iqbal *et al.,* 2019). To reduce the harmful effects of chemical fertilizers on human health and enviroment, nowadays there is increasing trend on the utilization of biofertilizer (Kasim *et al.,* 2019). Biofertilizers are usually prepared as carrier-based inoculants containing effective microorganisms (Wang *et al.,* 2009). One of the roles of microorganism in biofertilizer is phosphate solubilizer (Barus *et al.,* 2018).

Mangrove is one of potential ecosystems to isolate phosphate solubilizer microbes. Many microorganisms have been recognized for their ability to transformations of the nutrients, mainly N, P and K, to a form absorbable by plants, through nutrient decomposition and cycling of nutrients. Within these capabilities of the microorganisms are involved the mechanisms of production of organic and inorganic acids, chelation, ionic exchanges, enzymes and others (Pratiwi *et al.,* 2013; Sinatryani *et al.,* 2014; Da Silva *et al.,* 2017).

Phosphorus is one of the main limiting nutrient for plant growth because of its adsorption chemistry and formation of mineral complexes with different elements such as Ca, Al and Fe making phosphate immobile in soil (Shen *et al.,* 2011; Naibaho *et al.,* 2019; Silitonga *et al.,* 2019). Plants use different strategies to acquire P including exudation of organic anions, plants root associations with microorganisms such as phosphate solubilizing bacteria and

fungi (Sarabia *et al.,* 2018).

Indonesia has one of the highest microorganism biodiversity in the world. Yet, only a few of them have been discovered. So far, the studies about this topic only focus on bacteria and mold as biofertilizer consortium, but study on yeast still a few, and need to study further (Shen *et al.,* 2011). So, in this research we will isolate and identify phosphate solubilizer yeast from mangrove in Jenu Tuban, Indonesia.

Materials and Methods

Sample collection and Isolation

Soil samples (up to 20 cm. Depth) were collected from rhizosphere of *Rhizophora sp.* at Mangrove Center Tuban East Java. Soil samples were collected in sterile Ziploc plastics from each location, then mixed. The composite samples were moved to the laboratory for isolation and identification process (Das *et al.,* 2019).

Twenty five gram of soils was suspended in 225 mL of sterile, demineralized water, serially diluted and plated on Yeast Malt Extract Agar (YMEA) supplemented with chloramphenicol. Plates were incubated at 30 °C for 3 days. Colonies were identified based on macro-morphological types using magnifier and binocular microscopy. Each colony type were then purified without punctuation point (Masinova *et al.,* 2018).

Potency Assay

The yeast were screened on Pikovskaya's agar medium with the spot inoculation technique and incubated at 30 °C for 5 days. The quantitative estimation or abilities of the phosphate solubilizing yeast was determined in terms of *Phosphate Solubilizing Indexes* (PSI). *Phosphate solubilizing* indexes (PSI) was calculated using by formula from Alam *et al.* (2013).

Yeast Identification

The colony morphology of the isolated yeast were examined after grown on Yeast Malt Extract Agar medium at 30 °C for 3 days and its colony morphology, pigmentation, shape, size, elevation, margin, and appearance of the colnoy surface were observed using magnifier (hand lens). The yeast were stained using Lactofenol Blue and observed under a binocular microscope (1000x magnification).

The yeast were cultivated on Yeast Malt Broth medium and incubated for 2 days. Yeast growth in liquid medium may result in the formation of a compact, coherent, flocculent or mucoid sediment, a ring, islets or a pellicle. Ascospore was stained using the Schaeffer-Fulton's modification method. Capsule was stained using violet crystals for 5 minutes and $CuSO_4.5H_2O$ (Copper sulfate) (Kreger-van, 1987). For urease test, sugar fermentation test, and citrate assimilation test were used MICROBACT™ GNB 12A/B OXOID identification kit consist of 24 microplates with biochemical substrates. Positive

Table 1. Colony morphology of 17 yeast isolates

Yeast	Morphology Colonies
K1	White, circular 0.21 cm, convex, entire with hair-like formation, dull
K2	White, circular 0.22 cm, convex, entire with hair-like formation, dull
K3	Cream, circular 0.14 cm, convex, entire with hair-like formation, dull
K4	Cream, circular 0.15 cm, convex, entire with hair-like formation, dull
K5	White, circular 0,21 cm, convex, entire with hair-like formation, glistening
K6	White, circular 0.2 cm, convex, entire with hair-like formation, glistening
K7	White, circular 0.14 cm, umbonate, entire with hair-like formation, dull
K8	Cream, circular 0.21 cm, convex, entire with hair-like formation, dull
K9	White, circular 0.13 cm, convex, undulate with hair-like formation, dull
K10	White, circular 0,21 cm, convex, undulate with hair-like formation, dull
K11	Cream, circular 0.13 cm, convex, entire with hair-like formation, glistening
K12	Pink, circular 0.05 cm, convex, entire, glistening
K13	White, circular 0.24 cm, convex, Filamentous with hair-like formation, dull
K14	White, circular 0.04 cm, convex, entire with hair-like formation, glistening
K15	Cream, circular 0.11 cm, convex, entire with hair-like formation, glistening
K16	White, circular 0.12 cm, convex, entire with hair-like formation, glistening
K17	White, circular 0.04 cm, convex, entire, glistening

results from the test was produced discoloration in microplates. The data was analyzed with OXOID handbook and The Yeast a taxonomic study.

Results and Discussion

A total of 17 yeasts strains were grown on Yeast Malt Extract Agar medium. The yeast were identified based on their colony morphology depending on its pigmentation, shape, size, elevation, margin, dan appearance of the colony surface. The following table summarizes the result (Table 1).

Table 2. Phospate solubility index of 17 yeast isolates

No	Colony Isolate Code	Colony Zone (cm)	Clear Zone (cm)	Phosphate solubility index
1	K1	3.5	12.3	2.55
2	K2	4	12.3	2.07
3	K3	4.1	11.7	1.87
4	K4	4.2	13.3	2.17
5	K5	4	12	2
6	K6	5	12.7	1.53
7	K7	4.5	12.4	1.73
8	K8	4	13.9	2.48
9	K9	4.7	12.9	1.73
10	K10	3.6	12.0	2.31
11	K11	4.6	12.7	1.77
12	K12	3.7	11.5	2.11
13	K13	4.5	11.5	1.54
14	K14	3.1	11.2	2.58
15	K15	4.3	12.1	1.82
16	K16	4.8	8.9	0.86
17	K17	4.1	8.3	1.04

A total of 17 yeasts were evaluated for their phosphate solubilization activity on Pikovskaya's agar. Among those potency assay, seventeen yeast isolated were positive for phosphate solubilization (Table 2), but only three highest potential isolates were K14, K1 and K8. Their phosphate solubilizing indexes are 2,58, 2,55, and 2,48 cm within 5 days of incubation (Figure 1).

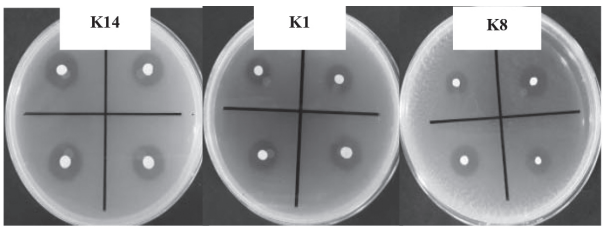

Fig. 1. Isolates with the highest phosphate solubilizing activity on pikovskaya's agar

Three highest yeast isolates evaluated for their phosphate solubilization ability on pikovskaya's agar, only one yeast isolates K14 were identified (Figure 2).

Colonies on Yeast Malt Extract Agar medium are circular 0.04 cm, convex, entire with hair-like formation, white and glistening. After 2 days in YM broth at 30 °C, cells are ellipsoidal to short cylindrical (3 x 4 µm) and occur singly, in pairs or in short chains, a thin powdery pellicle and sediment may be present, aerobic growth is white to cream (Fig. 2). After 1 week in Dalmau plate culture Corn Meal Agar medium, pseudohyphae consisting of branched chains of elongated cells are visible. True hyphae may be present, chlamydospores are absent. Capsule and ascospore are absent. Glucose and lactose fermentation are absent, Sucrose and raffinose fermentation are present. Citrate assimilated, sodium nitrate and nitrite are not utilized as sole nitrogen sources. Urease-negative. Kurtzman and Fell (2006) showed that morphological and physiological characteristics the phosphate solubilizing yeast isolate was identified as *Candida* genus.

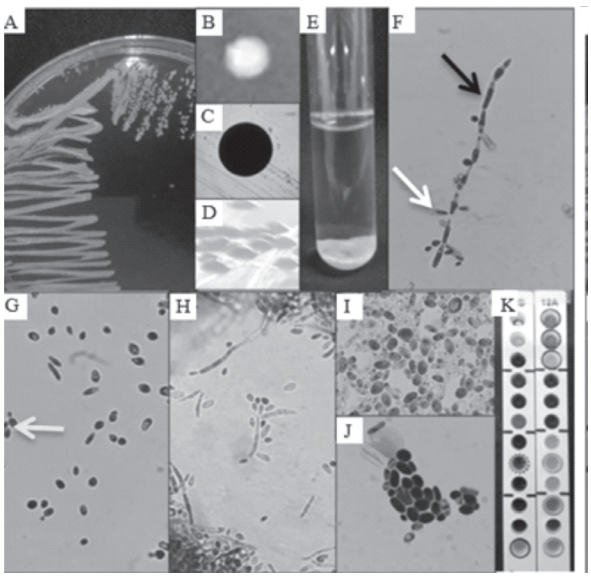

Fig. 2. Morphological and physiological characteristics of K14 isolates (**A**. Pigmentation on YMEA medium, **B.** shape on YMEA medium, **C.** margin on YMEA medium, **D.** elevation on YMEA medium, **E.**Yeast growth on YMB medium, **F.** pseudohyphae is shown by black arrows, blastospores shown by white arrows, **G.** multilateral budding shown by yellow arrows, **H.** Pseudohyphae on Corn-Meal Agar medium, **I.** ascospore test, **J**. Capsule test, **K.** urease test, sugar fermentation test & citrate assimilation test).

Al Fatih (2005) reported yeast belonging to *Candida tropicalis*, *Geotrichum candidum*, *Geotrichum capitatum*, *Rhodotorula minuta* and *Rhodotorula rubra*were phosphate solubilizing. Zerihun with (2017) reported the largest solubilization indexes recorded by *Phichia norvegensis* 3.35, *Cryptococcus albidus* var aerius 3.2, *Candida etchellsii* 2.9.

Conclusion

There are seventeen isolates of phosphate solubilizing *yeast* from Mangrove Center Jenu Tuban soil and three highest potential isolates were K14, K1, and K8 with *Phospate Solubilizing Indexes* (PSI) respectively 2,58 cm, 2,55 cm, and 2,48 cm. K14 was identified as a member of genus *Candida*.

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