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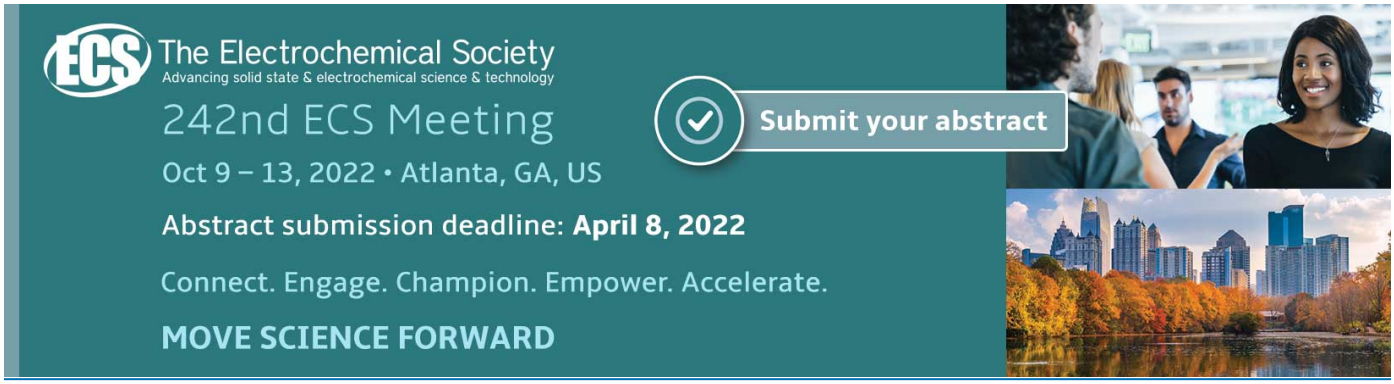

Caffeine-degrading endosymbiont fungi isolated from *Hipothenemus hampei.* Ferr as pre-analysis caffeine tolerance ability of coffee berry borer

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Abstract. The Coffee Berry Borer (CBB), *Hypthenemus hampei* Ferr., is the most destructive pest of coffee berry due to its ability to bore and complete their entire life cycle inside of the coffee berry. The detoxification mechanism of CBB ability to lives inside of coffee berry containing high natural insecticide compound such as caffeine is a very interesting study and it may lead to be an advantaged for CBB controlling methods. Isolation and screening of endosymbiont fungi from CBB using 0.5% caffeine plate agar as a minimum nutrient as well as a selective medium were obtained 10 isolates consist of 6 isolates of yeasts and 4 filamentous fungi which positively degraded caffeine content through their formed diameter of the colony. The presenting of these several caffeine-degrading endosymbiont fungi could be as pre-explanation about the caffeine tolerance mechanism of CBB.
Keywords: *caffein-degrading endosymbiont fungi, H. hampei, isolation, screening*

1. Introduction

Indonesia is the third bigger coffee bean producer in the world after Brazil and Vietnam at 2012 with total production approximately to 784 thousands including 601 milion tons (80.4%) of robusta coffee (*Coffea canephora*) and 147 milion tons of arabica coffee (*C. arabica*) [1,2]. However, recently Indonesian coffee production is decreasing gradually for several years namely 740 thousand tons (2013), 712 thousand tons (2014), 550 thousand tons (2015), 664 thousand tons (2016), 669 thousand tons (2017), and 674 thousand tons (2018) as well as lead to Indonesia at fourth position after Colombia1. The decreasing of coffee bean production may due to presenting of coffee bean borrer (CBB) *Hyphotenemus hampei* Ferr.

CBB is a most destructive pest in coffee bean. This insect has ability to bore coffee bean, use it as subtrate for their eggs and complete their life cycle inside it even its contains some toxic compound such as caffein, polyphenol and tannin. Feeding activity of CBB lead to decreasing of quantity and quality of coffee bean production until 40% [3,4,5]. The ability to grow well on extreme substrate such as caffeine can be as indicator that this insect possess high adaptation and tolerance ability with toxic medium. It may due to presenting of endosymbion microorganism such as bacteria and fungi inside of digestive system of *H. hampei*. Based on these background above, isolation and determination of caffeine- degrading endosymbiont fungi are observed here.

2. Materials and Methods

2.1 Sample Collection of H. hampei

Appropriately 15 sample of *H. hampei* were collected from coffee plantation around of State Polytechnic of Jember. All of *H. hampei* was taken from inside of coffee bean and allowed for 2 hrs without any feed appropriately coffee bean before fungal isolation procedure.

2.2 Isolation and Screeaning of Caffein Degrading Endosymbion Fungi

Sample of *H. hampei* was washed by 1 ml NaCl 0,85% 1 minute and then sterilazed by sodium hipocholare (NaOCl) 0.5% for 5 minutes. The sample was washed again by steril H2O with 3 times of replication. The steril 0.85% NaCl solution was used as washing solution to remove remaining spore on cuticule. Total 20 µl of washing water was inoculated into PDA plate + antibiotik streptomycin as negative control as well as to ensure the presence of remaining microorganism from cuticule. Then, the

3. Result and Discussion

Based on isolation results, there were 5 filamentous fungi and 9 yeasts from inside of CBB (Figure 1). All of these fungi were tested into caffeine medium to determine the caffeine- degrading ability using semi-quantitative method. The semi-quantitative caffeine degradation assay using 0.5% caffein + M9 medium was obtained 10 caffeine-degrading fungi positively (Figure 2a and 2b). Several isolated fungi could grew well on this caffeine medium and some of them couldn't. The un-growing fungi may due to these fungi have no degrading activity but can develop their tolerance mechanism inside of caffeine medium.

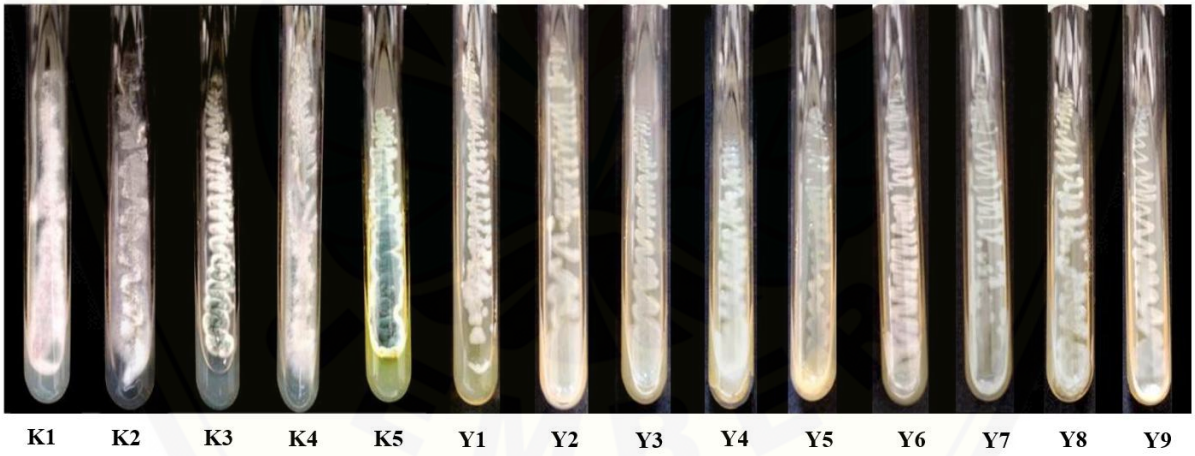

Figure 1. The potency of Caffeine-Degrading Bacteria on M9 media contains 1 g/L caffeine at 3 days incubation

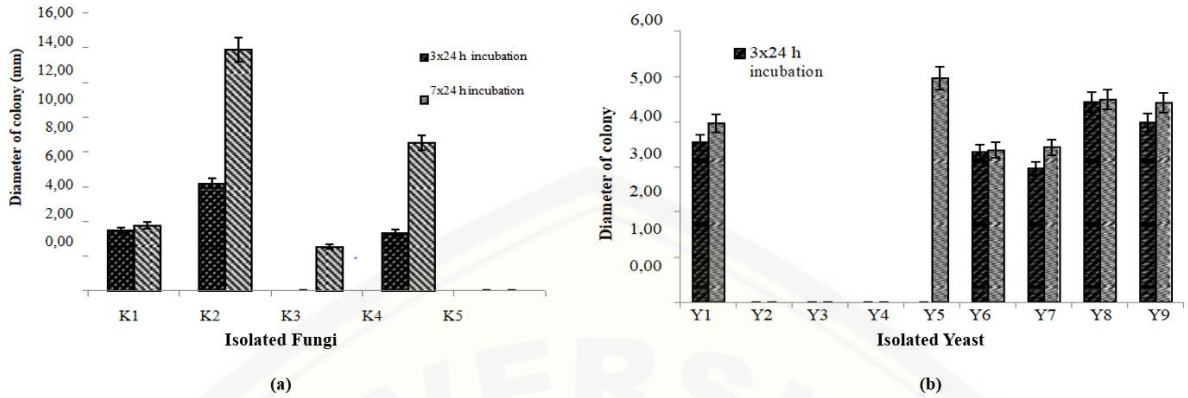

Figure 2. Growth patterns of isolated fungi on 0.5% caffeine agar medium. (a) Filamentous fungi growth diameter. (b) Isolated yeasts growth diameter

The growth pattern of isolated filamentous fungi and yeasts on minimal medium containing 0.5% caffeine + M9 medium were slowly compared to common culture medium such as PDA or YMEA. Based on morphological characteristic, the isolated filamentous fungi produced thin hyphae with small colony formed, while isolated yeasts needed more incubation time to enlarge their colony zone. The growing isolated fungi on minimal medium such as caffeine could be as preliminary study that these fungi are not only tolerance but also have caffeine-degradating activity since the caffeine medium has only sole carbon sources which comes from caffeine compounds ($C_8O_{10}N_4O_2$) This observation can be explaination how *H. hampei* survival mechanism inside of extreme growth environment to be.

4. Conclusions

There were several caffeine-degrading endosymbiont fungi isolated from *H. hampei* consist of 6 yeasts and 4 isolates of filamentous fungi. These isolated fungi were not only tolerance but also could be degraded the caffeine content inside of medium.

5. References

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