# TEST OF LIGNIN AND CELLULOSE DECOMPOSITION AND PHOSPHATE SOLUBILIZATION BY SOIL FUNGI OF GUNUNG HALIMUN

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**ABSTRACT**

In order to know the capability of lignin and cellulose degradation and phosphate solubilization by soil fungi of Gunung Halimun National Park, a study was carried out to qualitatively analyse its physiological properties. Out of 35 soil fungi tested, 1 species that belonged to Basidiomycetes degraded lignin, 32 species degraded cellulose, and 31 species dissolved inorganic phosphate. The presence of soil fungi that degraded cellulose and dissolved phosphate could be used as a candidate for biotechnology application as well as to increase soil fertility.

Key words: Cellulose, decomposition, phosphate, soil fiingi, solubilization.

## INTRODUCTION

Lignin is the major component of organic compounds in soil originated mainly from plant and animal debris. According to Orth *et al.* (1993), lignin is a random phenylpropanoid polymer which is difficult to degrade because of its complex, heterogenous structure. However, selected microorganisms are able to decompose high moleculer weight of lignin, phenolic compounds, or its partial decomposition products. One of these called Basidiomycetes has received attention because their ability to use lignin as the carbon and energy source.

According to Fikrinda *et al.* (2000), cellulose is the largest compound on earth, composed of 8000 - 12000 units of glucose that connected by p-l,4-glucoside bond. P-1,4-glucoside bond of cellulose fibre can be broken into monomer glucose by cellulose, namely a system of enzym composed of main three enzyme type such as endo-l,4-(i-glucanase, ekso-l,4-p-glucanase or cellobiohydrolase; and P-D-glucosidase.

The availability of phosphate in acid soil is low, because phosphor is adsorbed in the form of $A1PO_4$ and $FePO_4$, whereas in alkaline soil, phosphor will be adsorbed as $Ca_3(PO_4)2$. Plants can not absorb these forms for their growth. Soil phosphate to be available for plant roots or microbes should be hydrolized by secretion of organic acids that produced both by plants and microbes.

We isolated 35 fungal species from the soil samples of Gunung Halimun National Park (GHNP), that is the largest conservation area of tropical rain forest in Java. Many such studies were carried out in the same place, as those of Suharna (2000) and Suciatmih (2001). However, verification on the role of soil fungi on degradation of cellulose and lignin, and phosphate solubilization in this area has not been reported, therefore hydrolysis test of those subtances by soil fungi that collected at three altitudes of gunung Halimun might provide better information on their ecological role, and finally the possible use of soil fungi for biotehnology application and to increase soil fertility will be discussed.

## MATERIALS AND METHODS

### Test of Lignin Degradation.

To verify the ability of fiingi to hydrolize lignin, each soil fungi (35 species) was grown in petridish containing media as suggested by Paterson and Bridge (1994), and incubated at room temperature (27°-28° C). Four replicates were taken in each case. Fungi capable to hydrolize lignin is indicated by formation of clear zone surrounding growing colony.

**Test of Cellulose Degradation**

To investigate the capability of fungi to hydrolize cellulose, each soil fungi (35 species) was grown in petridish containing Whatman powder media, and incubated at room temperature (27°-28°C). Four replicates were taken in each case. Paterson and Bridge (1994) informed that fungi are capable to hydrolize cellulose when their colonies are surrounded by clearing zone.

**Test of Phosphate Dissolution.**

To discover the capability of fungi to hydrolize phosphate, each soil fungi (35 species) was grown in petridish containing Pikosvkaya media and incubated at room temperature (27°-28° C). Four replicates were taken in each case. According to Katznelson and Bose (1959), Sundara Rao and Sinha (1962), and Das (1963), the clearing zone of media indicates fungal capability to hydrolize phosphate.

**RESULTS**

Out of 35 species of soil fungi tested, only 1 species of soil fungi belongs to Basidiomycetes was able to hydrolize lignin (Table 1). This basidiomycete fungus hydrolized lignin and cellulose, and dissolved phosphate. This fungus was only isolated at 1000 m asl. (Table 2).

Out of 35 species of soil fungi tested, 32 species degraded cellulose. *Aspergillus* sp.2, *Gongronella butleri* and *Penicillium* sp.4 (see Table 1) did not degrade cellulose. Out of all isolates obtained at each altitude, 700 m (10 species), 1000 m (14 species), and 1500 m (16 species), there were 9, 13, and 15 species of soil fungi respectively, solubilized cellulose (Table 2). Cellulose degradation was started on 1 - 11 days after incubation. In general, zonation formed on 2-4 days after incubation.

Similar to cellulose hydrolysis, at every altitude also found soil fungi that dissolved Ca-phosphate. Amount of phosphate solubilizing fungi that found both at 700 m and 1000 m asl. was similar, namely 10 species, whereas at 1500 m asl. were 16 species (Table 1). Clear zone formation was observed quicker, generally it formed on 1 day after inoculation. Species of soil fungi which did not dissolve phosphate were *A. oryzae*, *C. echinulata*, *Penicillium* sp. 8, and unidentified 2. Two species of *Acremonium*, *Chaetomium* sp., *Cladosporium* sp., four species of *Eupenicillium*, *Mortierella* sp., *Paedlomyces* sp., two species of *Trichoderma*, unidentified 1, unidentified 3, and unidentified 4 were fungi both degraded cellulose and phosphate.

**DISCUSSION**

Out of 35 species of soil fungi tested, only 1 species of soil fungi which belongs to Basidiomycetes was able to hydrolize lignin (Table 1). It is different with observation by Artiningsih *et al.* (1999). They found that not only Basidiomycetes degraded lignin, but also *Paedlomyces* sp. In our results showed that *Paedlomyces* sp. did not degrade lignin. This is probably due to different species. According to Soetedjo *et al.* (1991), Basidiomycetes is fungi responsible for wood degradation. Wood degradator fungi usually have capability of attacking lignin quicker than other fungi. Kerem *et al.* (1992) noted that, effective lignin degradator is Basidiomycetes fungi which cause white rot disease. Srinivasan *et al.* (1995) observed that white rot basidiomycete fungi produce three enzymes namely lignin peroxidase, manganese peroxidase, and laccase, where all of those enzymes play important role on lignin degradation.

According to Soetedjo *et al.* (1991), cellulose can be decomposed quickly by specific organisms that belongs to bacteria, fungi, Actinomycetes and lower class animal.

Member of *Aspergillus*, *Chaetomium* and *Penicillium* examined in the present study was also degraded cellulose in another study (Sastraatmadja, 1994). Yani and Djajasukma (1991) added that production of cellulose by *A. oryzae* on cassava medium was 0,080 unit/10 material. Many of cellulolitic fungi found in GHNP area was attractive, since they play role on the C-cycle (via CO2 production), and cellulose enzyme could be used in many biotechnology application (Busto *et al.*, 1995) such as saccharification of material containing cellulose, detergent, food industry, and process of pulp and paper waste (Akiba *et al*, 1995).

Solubilization of phosphate by member of *Aspergillus*, *Cladosporium*, *Eupenicillium*, and *Penicillium* is in accord with results reported by Suciatmih (1999). In this study *A. oryzae* was the fungus did not degrade phosphate. This is different with observation by Suciatmih *et al.* (2000). They pointed out that *A. oryzae* degraded phosphate with its zone formation was 1-6 days. It may be because of different variety. *Aspergillus flavus* examined in the present study was also degraded phosphate in another study (Suciatmih *et al*, 2000). Das (1963) added that some *Aspergillus* such as *A. awamori*, *A. flavus*, and *A. niger* besides degraded Ca-phosphate, they can also increase mineralization of organic phosphate. Many of phosphate solubilization fungi found, surely attractive. Besides it stimulate growth of plant, it is also possible to use these fungi to improve cultivated plants that undergoing P-deficient in alkaline soil. Pareek and Gaur (1973) and Subba Rao (1994) noted that phosphate solubilization caused by "nonvolatile organic acids" produced by fungi such as citric and oxalate acids. Increased of these organic acids usually followed by sharply decreased of pH, result in Ca-phosphate solubilization. According to Subba Rao (1982), besides of pH decreased, these organic acids will bind cations in the form of stable complex with Ca2+, Mg2+, Fe3+, and A13+, result in phosphate dissolution.

## CONCLUSIONS

Out of 35 species of soil fungal collections of gunung Halimun tested, 1 species of Basidiomycetes degraded lignin, 32 species degraded cellulose, and 31 species dissolved phosphate. *Aspergillus* sp.2, *G. butleri*, and *Penicillium* sp. 4 did not degrade cellulose, whereas *A. oryzae*, *C. echinulata*, *Penicillium* sp.8, and unidentified 2 did not dissolve phosphate.

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Table 1.Diversity of culturable soil fungi and its ability to hydrolize lignin, cellulose, and inorganic phosphate.

| No | Species of fungi | Zonation (day) | | |
|---|---|---|---|---|
| | | Lignin | Cellulose | Phosphate |
| 1. | *Aremonium* sp. 1 | - | 6 | 1 |
| 2. | *Acremonium* sp.2 | - | 3 | 3 |
| 3. | *Aspergillus flavus* | - | 7 | 1 |
| 4. | *A. otyzae* | - | 5 | . |
| 5. | *Aspergillus* sp. 1 | - | 7 | 1 |
| 6. | *Aspergillus* sp.2 | - | - | 1 |
| 7. | *Aspergillus* sp.3 | - | 5 | 1 |
| 8. | *Chaetomium* sp. | - | 2 | 3 |
| 9. | *Cladosporium* sp. | - | 3 | 3 |
| 10. | *Cunninghamella echinulata* | - | 8 | - |
| 11. | *Eupenicillium* sp.l | - | 2 | 2 |
| 12. | *Eupenicillium* sp. 2 | - | 8 | 1 |
| 13. | *Eupenicillium* sp.3 | - | 2 | 1 |
| 14. | *Eupenicillium* sp.4 | - | 3 | 1 |
| 15. | *Gongronella butleri* | - | - | 1 |
| 16. | *Mortierella* sp. | - | 3 | 1 |
| 17. | *Paecilomyces* sp. | - | 2 | 1 |
| 18. | *Penicillium* sp. 1 | - | 5 | 1 |
| 19. | *Penicillium* sp.2 | - | 11 | 1 |
| 20. | *Penicillium* sp.3 | - | 4 | 4 |
| 21. | *Penicillium* sp.4 | - | - | 1 |
| 22. | *Penicillium* sp.5 | - | 4 | 2 |
| 23. | *Penicillium* sp.6 | - | 4 | 1 |
| 24. | *Penicillium* sp.7 | - | 1 | 1 |
| 25. | *Penicillium* sp.8 | - | 10 | . |
| 26. | *Penicillium* sp.9 | - | 4 | 2 |
| 27. | *Penicillium* sp.10 | - | 1 | 1 |
| 28. | *Penicillium* sp. 11 | - | 1 | 1 |
| 29. | *Trichoderma* sp. 1 | - | 6 | 1 |
| 30. | *Trichoderma* sp. 2 | - | 4 | 3 |
| 31. | unidentified 1 | - | 4 | 5 |
| 32. | unidentified 2 | - | 4 | . |
| 33. | unidentified 3 | - | 2 | 1 |
| 34. | unidentified 4 | - | 2 | 1 |
| 35. | Basidiomycetes | 6 | 11 | 1 |

Table 2. Total of lignin and cellulose, and phosphate solubilizing fungi and soil pH at various altitude.

| Altitude (m) | pH | Total of fungi | Total of fungi | | |
|---|---|---|---|---|---|
| | | | Lignin | Cellulose | Phosphate |
| 700 | 6,10 | 10 | - | 9 | 10 |
| 1000 | 3,89 | 14 | 1 | 13 | 10 |
| 1500 | 3,50 | 16 | - | 15 | 16 |