TELAAH BIOKIMIAWI PROSES ASOSIASI
Shorea selanica DAN Scleroderma columnare:
SUATU PENDEKATAN BIOSINTESIS

Oleh:
LATIFAH KOSIM DARUSMAN

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SUMMARY

LATIFAH KOSIM DARUSMAN. Biochemical Study on the Association Process of Shorea selanica and Scleroderma columnare: A Biosynthetical Approach; supervised by Djoko S. Moeljohardjo (chairman), G.A. Wattimena, Siti Soetarmi Tjitosomo, Yahya Fakuara Ts, and Muhamad Wirahadikusumah.

Mycorrhiza is a symbiotic, non-pathogenic association of a fungus and the roots of a plant. This association could be searched either through the plant activities as a host, or the fungous activities as a mycosymbiont. This dissertation is based on the research mentioned above, which had been conducted from 1992 until 1995. The objectives of the research are: (1) to compare plant growth, nutrient proportion, specific activity and kinetic characteristics of acid-phosphatase in the rhizosphere area as a result of the inoculation of a mycosymbiont; (2) to discover secondary metabolites resulted from the association process in the mycorrhizal roots; (3) to compare the specific activity and kinetic characteristics of acid-phosphatase and chitinase, patterns of lipid, carbohydrate and protein; between the mycorrhizal and non-mycorrhizal roots; (4) to study the in vitro
antagonistic effects of *S. columnare* Berk. & Br. to pathogenic microbes. This research covered four experiments: preliminary research, nursery experiment in Darmaga, *in vitro* mycorrhizal synthesis and microbial study experiments in the Laboratory of Chemistry Faculty of Mathematics and Natural Sciences and in the Laboratory of Silviculture Faculty of Forestry. The preliminary research was conducted in order to find the suitable growth medium in terms of microbial growth and extracellular metabolite production. The nursery experiment was designed to analyze growth variables, root structures, antimicrobial compounds and plant growth regulators. The *in vitro* mycorrhiza synthesis experiment was conducted to prove the association in the *in vitro* condition and to find possible phytoalexin-analog compounds in the mycorrhizal roots. While microbial study experiment were conducted to test the antagonistic characteristics of mycorrhizal fungi, the antibacterial characteristics of extracellular metabolites, and the ability of the fungi to produce plant growth regulators.

*S. columnare* isolates were obtained from the basidiocarps collected from the Dipterocarp Forest at Haur Benten Experimental Station, while the *S. selanica* cuttings were prepared from the 1-year-old saplings under
the 40-year-old stand at Darmaga Experimental Station. In the preliminary research, *S. columnare* isolates were grown in Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Modified Melin-Norkarns (MMN) and Malt Extract (ME) media. In the nursery experiment, the soil used as a medium was sterilized by the \( \gamma \) ray from Co nucleus. There were four treatments in the *in vitro* mycorrhizal synthesis experiment: (1) control, (2) inoculated, (3) root exudate added and (4) inoculated and root exudate added. Instead of soil, vermiculite was used as a medium, and the MS liquid medium was prepared for nutrient source. PDA and MEA media and ME and MMN liquid cultures were used in the microbial study experiment. *Staphylococcus aureus* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *P. solanacearum* were used in the bacterial test, while *Fusarium sp.* and *Rhizoctonia solani* were used in the fungal test.

The preliminary research showed that both PDA and MEA media could produce good mycelial growth, but in terms of dry weight ME medium produced higher than PDA medium did. The highest antibacterial activity against *S. aureus* (ATCC 25922) was found in the hexane extract of PDA and MEA media, with the clear diameter zone (d) formed of 23.4 and 23.0 mm respectively.
The results of the nursery experiment showed that inoculation could improve the quality of plant growth with 18.08% height increment and 20.25% increase of root weight. The growth improvement also showed the increase of phosphate uptake, caused by 21.85% increase of activity and 27.25% maximum rate of root acid-phosphatase. Inoculation could also increase the content of phosphate in the stem of plants of 36 week after planting (wap) by 14.04%. While available phosphate in the soil was increased by 9.38% after the experiment was done. The increase of available P caused by the increase of activity and maximum rate of soil acid-phosphatase were 32.30 and 65.08%, respectively.

The improvement of growth due to the increase of IAA and IBA in the mycorrhizal root were 326.76 and 505.12%, respectively, and BTP identified from the mycorrhizal root was 0.077 µg/g root.

_S. columnare_ was shown to be a good symbiont for _S. selanica_, indicated by the infection rate of 84.5 \(\pm\) 4.2% and the increase of chitin content of 261.69%.

There were indications of macromolecular change caused by inoculation, although it was not significant (\(P > 0.05\)). Total lipid content was significantly increased by 27.96% and one characteristic polypeptide band could
be detected in the region of < 14,400 molecular weight, while the extract showed chitinase activity. Inoculation could change the properties of chitinase of the 16 wap (8 weeks after inoculation, wai) root, but the changes were not significant for the 36 wap (28 wai) root. Activity, specific activity, maximum rate and Michaelis constant at the 16 wap (8 wai) were increased by 12.72, 6.07, 116.23 and 37.90%, respectively; showed the existence of the uncompetitive inhibition of enzyme.

*S. selanica* and *S. columnare* could form association in the *in vitro* condition, this was shown by the formation of Hartig net and mycelial mantle, through structural analysis. There were radially elongated epidermis cells in the root of 28 wai, but these cell could not be identified in the root of 16 wai.

The plant-produced antimicrobial compound, identified by antibacterial activity of the root extracts showed that either control or inoculated of 28 wai root resulted some antibacterial activity against *P. solanacearum*. Non mycorrhizal root of 16 wai did not show the antibacterial activity. However, it was enhanced by the addition of root exudate to the rhizosphere area and reached the highest activity of 11.3 ± 1.3 mm zone (d) formed against *P. solanacearum*. This result showed the
indication of interaction between root exudate and fungal inoculation in producing the plant antimicrobial compounds. There was also indication of the role of flavonoid compounds in the association process. Flavanone, flavone, flavonol, malvidin, pelargonidin and cyanidin were identified from the root extracts.

S. columnare metabolites gave an antifungal activity against R. solani and antibacterial activity against P. solanacearum. The antifungal activity shown by the retardation of mycelial growth, for the ME and MMN butanol extracts were 56.81 ± 2.49 and 53.62 ± 4.13%, respectively. The antifungal compound was identified as one of pelargonidin or malvidin group. The strongest antibacterial activity found in the third fraction of PDA and MEA hexane extracts toward P. solanacearum with zone formed were 8.4 ± 0.7 and 13.4 ± 1.7 mm respectively. While antibacterial activity in the phenol fraction was shown in the third fraction from the MMN extract, with zone formed was 12.7 ± 0.1 mm. The antibacterial compound was identified as one of sesquiterpene and flavanone group. IAA, IBA and BTP could be identified from the extracellular metabolites of S. columnare grown in ME medium, with their concentration of 0.977, 0.326 and 0.011 ug/g extract, respectively. IAA was identified from the
extracellular metabolite of *S. columnare* grown in MMN medium with a concentration of 1.069 μg/g extract. There is indication that *S. columnare* could synthesize the IAA compound from tryptophan which acted as a precursor.
RINGKASAN

LATIFAH KOSIM DARUSMAN. Telaah Biokimiawi Proses Asosiasi Shorea selanica Dan Scleroderma columnare: Suatu Pendekatan Biosintesis; di bawah bimbingan Djoko S. Moeljohardjo sebagai Ketua; G.A. Wattimena, Siti Soetarmi Tjitrosomo, Yahya Fakuara Ts, dan Muhamad Wirahadikusmah sebagai anggota.

Mikoriza ialah suatu asosiasi simbiosis antara cendawan non patogen dengan akar tumbuhan. Asosiasi ini dapat ditelaah melalui aktivitas tumbuhan sebagai tanaman inang atau melalui aktivitas cendawan sebagai mikosimbi-on. Disertasi ini disusun berdasarkan hasil penelitian yang dilakukan dari tahun 1992 sampai 1995. Penelitian yang dilakukan bertujuan untuk (1) membandingkan tingkat pertumbuhan tanaman tersebut akibat inokulasi mikosimbion, proporsi hara, aktivitas spesifik serta karakteristik kinetik enzim fosfatase-asam di daerah rizosfer; (2) mencari metabolit sekunder hasil proses asosiasi pada akar bermikoriza; (3) membandingkan aktivitas spesifik dan karakteristik kinetik enzim fosfatase-asam dan kitinase, pola lipid, karbohidrat serta protein akar bermikoriza dan tidak bermikoriza; (4) mempelajari gejala antagonistik in vitro: S. columnare Berk. & Br. terhadap
mikroba patogen.


Isolat S. columnare berasal dari basidioma cendawan yang dikoleksi dari tegakan hutan Dipterocarpaceae di Kebun Percobaan Haur Bentes, Pusat Penelitian dan Pengembangan Hutan, Departemen Kehutanan. Setek pucuk S. selanica Bl. diambil dari anak dan yang berumur ± 1 tahun di sekitar pohon induk yang berumur ± 40 tahun yang ber-