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# **Science for Sustainable Utilization of Forest Resources in the Tropics**

**Midterm Report of JSPS-LIPI Core University Program  
in the Field of Wood Science during 1996-2000**



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# Biochemical Analysis of Organic Acid Metabolism of Symbiotic and Saprophytic Basidiomycetes Occurring in Forest Ecosystems

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## **Purpose of the Research Project:**

Forests and the wood they produce are among the Earth's most valuable natural resources. In our research project, we focus on an important role of fungi in carbon recycling in forest ecosystem on earth. Because recently a wide variety of symbiotic fungi have been receiving much attention on account of their tree-growth promoting effects, whereas wood-destroying fungi cause serious biological damage to timbers in service. Thus, if we succeed in elucidating biochemical mechanisms for their strategies to attack woods or promote tree growth, we can know how to control them for the benefits of the protection of wood resources against fungal attack to increase service-life of woods on one hand and to enhance growth of economically important timbers with aid of the symbiotic fungi on the other. Among these fungi are also economically important edible and medicinal mushrooms, some of which are grown industrially or can be hunted from natural forests and fields. However, reforestation is becoming a difficult task for economic target because of difficulty in investing money to slowly-growing tree plants in spite of recent serious deforestation in tropical countries. Therefore, if modern biochemistry and biotechnology succeed in growing a great deal of such economically important mushrooms in replanted forest areas, foresters are encouraged by mushroom production business as a sort of side-job from a socio-economical viewpoint. So, as a whole, there are three types of mushrooms or basidiomycetous fungi, such as wood-rotting, symbiotic, and edible fungi, we are interested in to investigate as described above. Surprisingly, however, even a very basic biochemical analysis of carbon metabolism for organic acids, which are extremely important for their life, have little been investigated.

During our collaboration work, we Japanese researcher decided to concentrate biochemical work on symbiotic and saprophytic fungi in laboratory on one hand and Indonesia researchers are encouraged to carry out the field work focussing on more practical and applied research led by Prof. Setiadi (IPB) and Prof. Madame Asmarlaili (USU). Because of shortage of time (3 years) and money (paucity) for conducting the collaborative research, we should like to focus on the exchange of academic information from different aspects throughout the collaboration work. However, the Ph.D. candidate graduate student (Erman Munir from USU) can take advantage of Monbusho scholarship to conduct the biochemical analysis with enzymes of the very important wood-destroying mushroom (*Fomitopsis (Tyromyces) palustris*) in this laboratory (WRI, Kyoto). We would like to summarize our recent new findings below.

### Summary of the Result:

Since we started our collaborative work only one year ago, our work is still under way. Nevertheless, the Indonesian graduate student and us endeavored cooperatively to explore a new research field in the kingdom of basidiomycetes. Fortunately, we have discovered a novel metabolic cycles (short-cut TCA-, glyoxylate-, and oxaloacetate/acetyl CoA - cycles) of organic acids in relation to biosynthesis of oxalic acid (Fig. 1) which seems to play a pivotal role in the

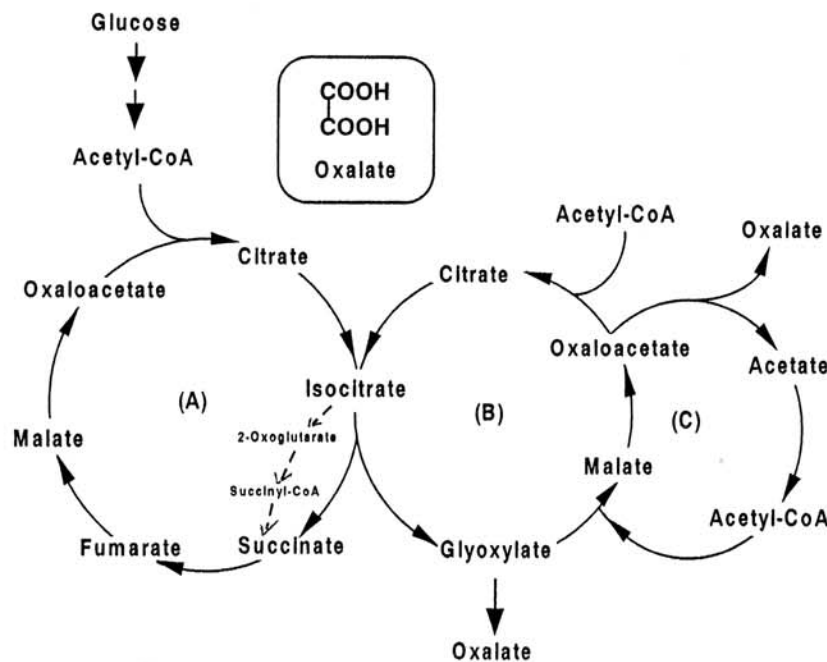


Fig.1. Novel Metabolic Cycle Systems for Biosynthesis of Oxalic Acid in a Copper-Tolerant Brown-rot Fungus *Fomitopsis (Tyromyces) palustris*.

fungal respiration and their energy acquisition or the growth. These types of fungi seem to expire predominantly oxalic acid instead of carbon dioxide which are expired by animals, plants and most microorganisms as a normal end product. In addition, we found for the first time that a number of wood rotting basidiomycetes have the Kornberg's glyoxylate cycle as a constitutive biochemical device to varying extents, in sharp contrast to the finding that the glyoxylate cycle only appears as an induced enzyme system only when microorganisms are grown on non-sugar substrates. Our first report has been accepted for publication in J, Wood Science (2001) as also included in this JSPS report. Furthermore, quite recently, we have succeeded in purifying the two key enzymes (isocitrate lyase (ICL) and malate synthase (MS) of the glyoxylate cycle from the same brown-rot or so called copper torelant fungus (*F. palustris*) as shown in Tables 1 and 2.

Alternatively as to symbiotic fungi, we have succeeded in establishing the sandwich symbiotic cultivation system for ectomycorrhizal fungus *Laccaria amethystea* and its host plant *Pinus densiflora*. By use of the symbiotic cultivation system, we would like to elucidate whether short-cut TCA-, glyoxylate-, and oxaloacetate/acetyl CoA - cycles function as a major metabolic pathway for isocitrate in *L. amethystea* during symbiosis with *P. densiflora*.

**Table 1.** Purification of Isocitrate Lyase (ICL) from the Brown-rot Fungus *F. palustris*.

Steps	Total Protein (mg)	Total Activity (units)	Yield (%)	Specific activity (units/mg)	Purification (fold)
Crude extract	481.0	197.0	100	0.41	1
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ppt. (20-70%)	320.1	184.0	93	0.57	1
TSK gel Phenyl-Toyopearl 650M	33.6	150.4	76	4.48	11
Protein-Pak DEAE 15HR	7.01	89.03	45	12.7	31
HiLoad Superdex 200	2.90	78.63	40	27.1	66
Cosmogel QA	1.46	45.36	23	31.1	76

**Table 2.** Purification of Malate Synthase (MS) from the Brown-rot Fungus *F. palustris*.

Steps	Total Protein (mg)	Total Activity (units)	Yield (%)	Specific activity (units/mg)	Purification (fold)
Crude extract	368.3	124.5	100	0.34	1
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ppt. (20-70%)	225.8	101.1	81	0.45	1
TSK gel Phenyl-Toyopearl 650M	25.0	62.08	50	2.48	7
Protein-Pak DEAE 15HR	8.95	40.95	33	4.58	13
HiLoad Superdex 200	0.161	15.89	13	98.70	290

## List of publications

E. Munir, J. J. Yoon, T. Tokimatsu, T. Hattori and M. Shimada: New role of the glyoxylate cycle enzymes in wood-rotting basidiomycetes in relation to biosynthesis of oxalic acid, *J. Wood Science*, in press (2001)

E. Munir, T. Hattori and M. Shimada, Distribution of isocitrate and malate synthase among wood-rotting fungi, *Proceedings of the Third International Wood Science Symposium*, 380-385, Nov., 2000, Kyoto, Japan

1