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In vitro Assay of Isoterpenoid Biosynthesis Using Cytosol and Microsomal Fractions of Pepper Suspension Cells

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Objectives

To understand isoterpenoid biosynthesic pathway which involved in the resistant of *Phytopthora* disease in red pepper cells under elicited condition.

Materials and Methods

1. Plant materials: suspension cell of red pepper and seedings.

2. Elicitation: Cellulase, Jasmonic acid

3. Assay method: *In vitro* reaction using H^3-labelled FPP with cytosol or microsomal fraction of pepper cells, infiltration of elicitors, TLC and scintillation count of the fraction

Results and Discussion

In vitro reactions of H^3-labelled fanesyl pyrophosphate (FPP) with crude enzymes of cytosol and micrsomal fraction in the elicited or control cells were conducted. FPP was converted into squalene by *in vitro* reaction of H^3-FPP with microsomal fraction in the control cell, and converted into 5-epi-aristolochene (5-EAS) by the reaction with cytosol fraction in the elicited cell. Capsidiol which is final product of sesquiterpenoid pathway from FPP, was *in vitro* synthesized by the reaction of FPP + cytosol+microsomal fraction from elicited cells. This result suggests that pepper cell posses a biochemical pathway to produce capsidiol under elicited condition (Figure 1). Extracellular capsidiol in the medium of suspension cultures was absent from control cells, but accumulated in the elicitor treated cells with cellulase or jasmonic acid. Infiltration of elicitors, cellulase or jasmonic acid, to the surface of leaf or fruit, stimulated the elicitation of the cells which resulted in the production of capsidiol and expansion of pathogene-like lesion around the elicitor treated region.

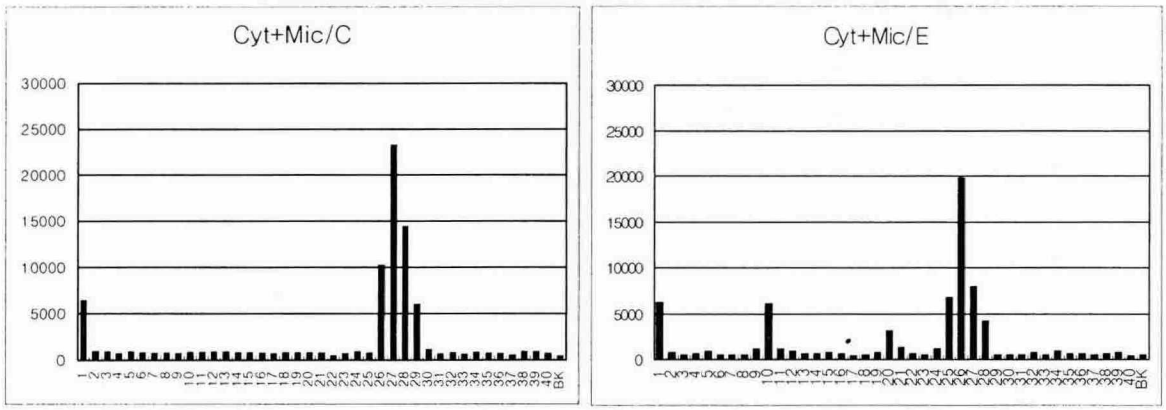

Figure 1. DPM of *in vitro* reaction product, capsidiol (Fr. 10), 5-EAS (Fr. 26), and squalene (Fr. 27), on TLC plate.

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