E227 Antifungal Agent Production by Co-culture of Plant Cell and Fungal Elicitor

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In order to antifungal agent production effect by co-culture fungal elicitor and plant cell were investigated. Fungal elicitor prepared from *Fusarium.solain F.oxysporium, F.moniliforme, P.capsici* and *R.solani. F.moniliforme* elicitor was the best in enhancement of antifungal agent between the fungal elicitor tested. Because it induced in quantities polysaccharide. Cabbage callus cell expressed high levels of the antifungal activity among the plant cell. The optimum time and pH of treated *F.moniliforme* elicitor for antifungal agent production were 48hours to 168hours and pH 5.8±0.1 in medium. Antifungal activity was detected in the crude extracellular solution, which was expressed as units/ml crude solution, was in descending order of 168hours > 144hours and 126hours > 96hours. The accumulation of antifungal activity during drought stress was confirmed by enzyme activity.

E228 Fumarase at other than mitochondria : Participation of extracellular fumarase in utilizing malate in carrot cells

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Carrot suspension cells exhibited an unique mode utilization of single carbon source malate. Malate, a frequently occurring organic acid in vascular tissues and apoplastic spaces, were investigated how cells use the externally added organic acid. Results showed that malate was not able to transported into cells while it was consumed rapidly to support cell growth. It turned out that malate was converted externally into fumarate which subsequently transported into cell. Fumarate level in medium increased during growth. Uptake rate of fumarate was accordingly induced and it was pH sensitive. An immunoblot experiment using fumarase antibody raised against *Arabidopsis* mitochondrial fumarase showed that fumarase appeared in medium to catalyte the extracellular conversion of malate into fumarate. Activity of fumarase was also induced both in medium and cells. Activities of malic enzyme and malate dehydrogenase was also induced in cells. The pHs in medium and cells were acidified and alkalized, respectively, as cells grew on malate. These results strongly suggest that carrot cells operate a following metabolic steps in utilizing malate; extracellular conversion of malate into fumarate-fumarate uptake-fumarate conversion into malate (reverse reaction of fumarase) alkalizing cytosol-malate metabolism into oxaloacetate and/or pyruvate. To the best of our knowledge this study presents the first evidences of physiological occurrence of fumarase outside of mitochondria. It also provide at first strong evidence in plants in that uptake of a substrate are coupled to metabolic pathways