

## Characterization of Expressed Sequence Tags from *Centella asiatica* (L.) Urban Hairy Roots Elicited by Methyl Jasmonate

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### Objectives

*Centella asiatica* (L.) Urban contains triterpenoid saponins such as centella saponin, asiaticoside, madecassoside and sicefollenoside (Kartnig and Hoffmann-Bohm 1992; Matsuda et al. 2001). To identify the genes involved in biosynthesis, an expressed sequence tags (ESTs) analysis was performed from *C. asiatica* hairy roots after treatment of methyl jasmonate (MeJA) as an elicitor. We analyzed the gene expression levels of glycosyl transferase (GT) and cytochrome P450 using real-time RT-PCR.

### Materials and Methods

#### ○ Plant materials

A *C. asiatica* hairy-root culture was generated by infection with *Agrobacterium rhizogenes* strain R1000. Induced hairy roots were cultured on hormone-free half-strength MS medium at 23° C in darkness. After 5 weeks of pre-cultivation, 0.1 mM MeJA was introduced to the medium.

#### ○ Sequencing, assembly and annotation

Poly(A)+ mRNA were isolated and a cDNA library was constructed using Uni-ZAP XR vector (Stratagene). cDNA clones were sequenced with T3 primers for the clones' 5' region analysis. ESTs were clustered and assembled using the Phrap DNA assembly program (<http://www.phrap.org>). The clustered sequences (contigs) were compared to non-redundant protein databases using BLASTX searches. Functional annotation of putative proteins from the ESTs was also conducted using the Gene Ontology (GO) database (<http://www.geneontology.org>).

#### ○ Real-time RT-PCR analysis

Total RNA was extracted from *C. asiatica* hairy roots after 0 h, 12 h, 24 h, 48 h, 3 day, 5 day, and 7 day of MeJA treatment. Two µg of total RNA was used for first strand cDNA preparation and the cDNA was subjected to real-time RT-PCR. SYBR Green master mix (Invitrogen, Carlsbad, CA) was used for quantification and  $\beta$ -actin was used as internal control.

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## Results

- A cDNA library was constructed from MeJA treated *C. asiatica* hairy roots.
- Sequencing of 4,896 cDNA clones generated 4,358 5' -end high-quality ESTs and grouped to 2,643 clusters.
- A BLASTX analysis showed that 2,068 of these genes were similar to genes present in the NCBI database.
- A total of 225 genes were most similar to *Hedera helix*.
- Many of GT and cytochrome P450 were found and some of them were induced after 12 hour of MeJA treatment from *C. asiatica* hairy roots

Table 1. The frequent contigs from the *C. asiatica* hairy root ESTs.

Sequence ID	No. of Seq.	Accession	Description	Identity (%)	E-value	Organism
ROTLCD001_10-B09	225	AAO38031.1	delta12-fatty acid acetylenase	85	1E-145	<i>Hedera helix</i>
ROTLCD001_18-F03	209	AAG23930.1	ELI7.9	91	1E-152	<i>Petroselinum crispum</i>
ROTLCD001_47-E10	105	AAL47574.1	thaumatin-like protein	62	4E-74	<i>Daucus carota</i>
ROTLCD001_49-A04	103	AAF80560.1	omega-6 fatty acid desaturase	63	1E-110	<i>Sesamum indicum</i>
ROTLCD001_08-C02	55	AAM21199.1	pathogenesis-related protein 5-1	85	1E-108	<i>Helianthus annuus</i>
ROTLCD001_46-C01	44	ABG29599.1	gyrase inhibitor	96	3E-49	<i>Escherichia coli</i>
ROTLCD001_07-F11	41	P80889	Ribonuclease 1	70	7E-57	<i>Panax ginseng</i>
ROTLCD001_09-F03	35	ABN03967.1	acidic chitinase	68	2E-92	<i>Gossypium hirsutum</i>
ROTLCD001_15-G06	35	ABY60454.1	putative polyubiquitin	100	2E-83	<i>Adonis aestivalis</i>
ROTLCD001_44-A03	32	CAA10232.1	metallothionein-like protein class II	72	3E-16	<i>Fagus sylvatica</i>
ROTLCD001_04-E02	29	NP_189274.1	GDSL-motif lipase/hydrolase family protein	52	1E-79	<i>Arabidopsis thaliana</i>
ROTLCD001_14-F12	24	ABS00393.1	cytochrome P450 hydroxylase	44	1E-55	<i>Hyoscyamus muticus</i>
ROTLCD001_07-F04	22	BAF80349.1	exo-polygalacturonase	59	2E-88	<i>Daucus carota</i>
ROTLCD001_11-E11	21	AAB50843.1	iEP4	53	3E-74	<i>Daucus carota</i>
ROTLCD001_02-H06	20	ABC01905.1	fructose-bisphosphate aldolase-like protein	91	1E-134	<i>Solanum tuberosum</i>

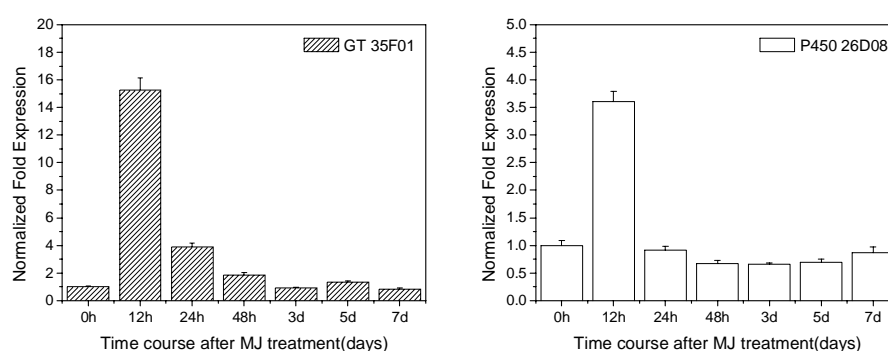


Figure 1. Relative expression patterns of GT 35F01 and cytochrome P450 26D08 genes of *C. asiatica* hairy roots elicited by MeJA. *C. asiatica* hairy roots generated using real-time RT-PCR as described in methods. The fold differences in the level of expression of respective cDNA in different time were presented as the mean of three independent experiments with respective SE.