

Galactinol Synthase Gene of *Coptis japonica* is Involved in Berberine ToleranceKojiro Takanashi*Laboratory of Plant Gene Expression, RISH, Kyoto University*

Many plant secondary metabolites show strong biological activities and potentially also toxic to plants, while plants producing those active compounds are usually insensitive to their own metabolites, suggesting that they have a species-specific detoxification mechanism. In order to clarify the detoxification mechanism of alkaloid, we used cultured cells of *Coptis japonica*, which are capable of producing a yellow isoquinoline alkaloid berberine and accumulate it in the vacuole, and show strong tolerance for berberine, unlikely to other plant cells that do not produce berberine. We established a fission yeast strain that was sensitive to berberine, with which a functional screening using *C. japonica* cDNA library was performed. One cDNA clone, which conferred clear berberine tolerance, encoded galactinol synthase (CjGolS).

GolS is known to catalyze the first committed biosynthetic step for raffinose family oligosaccharides (RFO). GolS utilizes UDP-galactose and *myo*-inositol as substrates to form galactinol, and this enzymatic reaction step reportedly plays a key regulatory role in the carbon flux branching between sucrose and RFO. To determine the reason for the berberine tolerance conferred by CjGolS, the berberine content in CjGolS-expressing yeast cells was measured by HPLC. The cellular levels of berberine in CjGolS-expressing yeast grown in 25 μ M or 50 μ M berberine-containing medium were not significantly different from the berberine levels in the control cells.

The expression levels of *CjGolS* in various organs of intact plant and cultured cell lines of *C. japonica* were analyzed by semi-quantitative RT-PCR analysis. The *CjGolS* gene was expressed in all parts of the plant, preferentially in the aerial parts, petioles, and leaves (Fig. 5A), where berberine was not produced or highly accumulated. Whereas, the steady-state level of *CjGolS* expression was higher in 156-S, which produces about 3-fold higher amounts of berberine as compared to CjY, a low berberine-producing line (Fig. 5B).

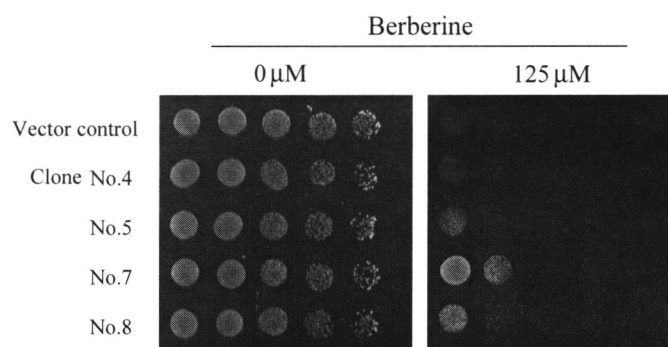


Fig. 1. Screening for Berberine-Tolerance Genes in *C. japonica* cDNA Library.

These panels show the 3rd screening round, in which the plasmid containing the berberine tolerance cDNA identified in the 1st and 2nd screening rounds was reintroduced into fission yeast as the 3rd screening round. Clone No.7, which encoded galactinol synthase (CjGolS), showed distinct berberine tolerance.