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Genetic engineering approaches to study of the opines of natural GMOs

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A few dozen naturally transgenic plants have been described to date [1]. Many of them contain DNA-sequences homologous to opine biosynthesis genes. Chemically, opines fall into two major structural classes: secondary amine derivatives and sugar-phosphodiesters [2]. The concentration of these molecules in the crude plant extract of nGMOs could be less than 1 pmol, which makes it difficult to study them. However, structure clarification of plant opines reveal their function in plants. The aim of our work is the development of protocol for large scale production, purification and definition of the chemical structure of plant opines.

The approach includes amplification of full-length opine synthase gene by PCR and cloning it using pENTR™/D-TOPO™ Cloning Kit (Thermo Fisher) according to its manual; subcloning in pDest527 using LR Clonase™ II Plus enzyme (Thermo Fisher) according to its manual; transformation NiCo21(DE3) chemically competent *E. coli* cells with obtained recombinant plasmids. After the confirmation of transgenic nature, the *E. coli* cells should be grown on medium with ampicillin 100 mg/l to an optical density at 600 nm 0.5–0.6. Then an equal volume of LB medium with 1 mM isopropylthio-β-galactoside (IPTG) should be added for induction opine synthesis. In case of synthesis of sugar-phosphodiesters, cultural media should also contain 0.02 M arabinose, glucose, and sucrose, respectively. Cells are cultivated during 4 hours for induction of opine synthesis. After that cells are pelleted by centrifugation for 10 minutes at 5000 rpm/min. The original strain and cells grown on the medium without IPTG are used as controls.

The cells are extracted with 80% methanol. The culture liquids are evaporated and redissolved in 80% methanol. The opines are separated by normal-phase chromatography using a CHROMABOND® Flash BT. Thin-layer chromatography is used to analyze the fractions, and to confirm the component opines-like compounds. High-resolution mass spectra are recorded on a Bruker micrOTOF 10223 mass spectrometer (electrospray ionization), eluent 80% MeOH. The ^{31}P NMR spectra are acquired on a Bruker Avance 400 spectrometer (400, 100, and 162 MHz, respectively). The ^{1}H spectra are analyzed in D_2O, with residual solvent signals (7.26 ppm for ^{1}H nuclei) as the internal reference. The ^{31}P NMR is measured relative to H_3PO_4.

The one phosphoric acid residue in the opine structure was confirmed by NMR spectroscopy.

We applied this method to characterize agrocinopine A-like compound in *Nicotiana* and propose it to produce opine-like plant compounds for further biochemical analysis.

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