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Poster Abstract - B.35

CLONING OF A BETA-AMYRIN SYNTHASE GENE (*OXA1*) FROM *ASTER SEDIFOLIUS* AND CONSTRUCTION OF AN EXPRESSION *AGROBACTERIUM* VECTOR

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Plants produce diverse classes of secondary metabolites that can be structurally divided into five major groups: polyketides, terpenoids, alkaloids, phenylpropanoids and flavonoids (Oksman-Caldentey and Inzè 2004. Trends Plant Sci 9: 433-440). In particular, triterpenoid saponins, produced mostly by dicotyledonous species, have an important role *in planta* for their antimicrobial activity. In addition, they can be interesting as pharmaceuticals. *Asteraceae* family is a good source of triterpenoid saponins, many of them being isolated from the genus *Aster s.l.* (tribe *Astereae*). A phytochemical analysis of leaves from *A. sedifolius* has led to the isolation of three novel triterpenoid saponins, named astersedifolioside A, B and C that showed *in vitro* antiproliferative activity against a thyroidal tumor cell line (Corea et al. 2004. Bioorg Med Chem, 12: 4909-4915). An approach of metabolic engineering in *A. sedifolius* based on the overexpression of a gene encoding beta-amyrin synthase, a key enzyme in the triterpene pathway, was established to increase the production of the astersedifoliosides.

Cloning of full-length triterpene synthase gene, *OXA1*, has been performed in *A. sedifolius* (GenBank AY836006). The enzyme encoded by *OXA1* catalyzes the cyclization of 2,3-oxidosqualene into tetracyclic and/or pentacyclic carbon skeleton for the biosynthesis of aglyconic molecule of the saponins.

For functional analysis of *OXA1* gene, it was transformed a yeast mutant which accumulates oxidosqualene inside the cell since lanosterol synthase activity is lacking and, consequently, the cyclization of 2,3-oxidosqualene blocked. The complementation of this mutant with *OXA1* is under investigation.

An expression vector useful for *Agrobacterium* transformation was constructed with *OXA 1* under the control of *CaMV 35S* promoter. For this purpose, two primers were designed at each end of *OXA1* to create Xba I site upstream ATG start codon and Sac I site downstream of the stop codon. At the present, strain LBA4404 of *A. tumefaciens* has been transformed with the construct *CaMV35S::OXA1*.