

Biochemical engineering of natural product biosynthesis pathways™

Metabolic engineering of natural products is a science that has been built on the goals of traditional strain improvement with the availability of modern molecular biological technologies. During the past decade, the state of the art in metabolic engineering of natural products research has been advanced from the first proof-of-principle experiment based on minimal known genetics to a fairly commonplace event using highly specific and sophisticated gene manipulation methods. With the availability of specific genes, host organisms, vector systems, and advanced molecular biological tools, **Avesthagen** is aimed at translating metabolic engineering into an industrial reality.

Avesthagen has been focused on the enhanced synthesis of therapeutically relevant secondary metabolites such as *colchicine* and *forskolin* in cell-culture systems of *G.superba* and *C.forskohlii* respectively. Callus cultures of *G.superba* were biolistically transformed with a homologue of the recently characterized **ORCA** family of transcription factors that have been shown to be involved with the

regulation of both primary and secondary metabolic flux in higher plant systems, so as to facilitate an enhanced, controlled production of *colchicine*. In similar lines, a homologue of the **R2/R3-myb** family of transcription factors has been over-expressed in *C.forskohlii* cultures to result in an increased accumulation of *forskolin*.

The research team at **Avesthagen** is currently analyzing the modulation in the levels of the aforementioned secondary metabolites in transformed callus cultures using high-sensitivity detection techniques (MALDI-TOF). Representative results of the same have been depicted in **Annexure I**.

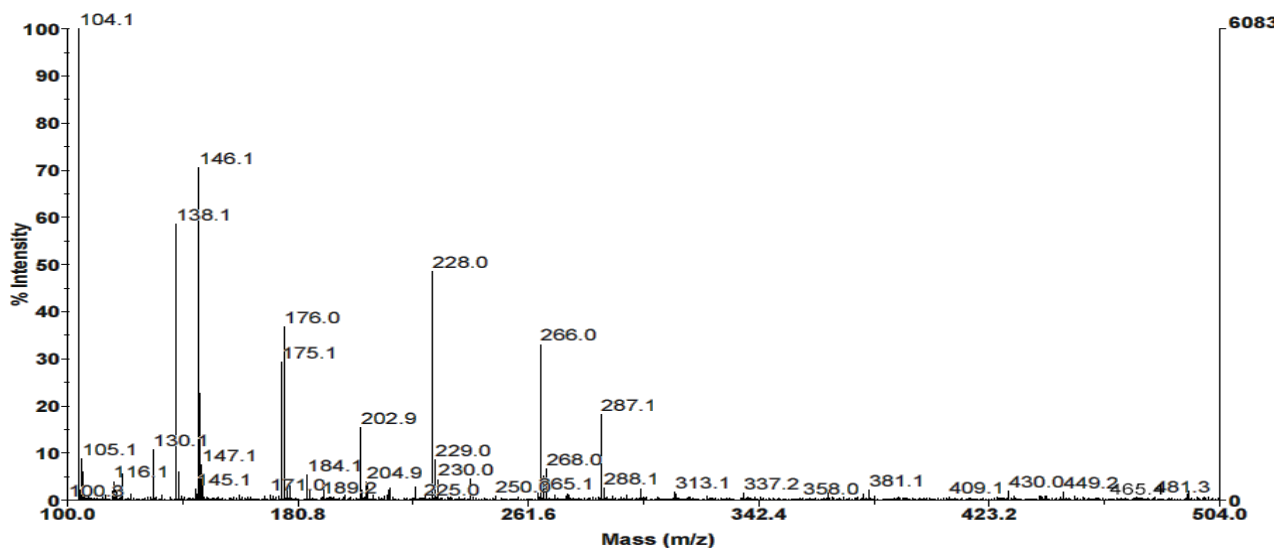
Reference:

- Verpoorte, R and Memelink, J. *Engineering secondary metabolite production in plants*. Curr Opin Biotechnol. 2002 ; 13(2):181-7.
- Memelink, J; Kijne, JW; van der Heijden, R and Verpoorte, R. *Genetic modification of plant secondary metabolite pathways using transcriptional regulators*. Adv Biochem Eng Biotechnol. 2001; 72:103-25.

Annexure I:

MALDI-TOF based comparative analysis of the *G.superba* transgenic calli, that were transformed with the ORCA-homolog transcription factor, showed detectable levels of colchicine and a few of its precursor molecules unlike in the case of the control calli. Thus the genetic modification of the *G.superba* calli with the ORCA-homolog has shown a directed metabolic flux both at the primary and secondary metabolite level.

MALDI-TOF profile of the methanolic extract of *G.superba* control callus:



MALDI-TOF profile of the methanolic extract of *G.superba* transgenic callus:

