Laboratory of Natural Product Biosynthesis Systems,

Dept. of Genetic Engineering, Institute of Cell Biology and Genetic Engineering

Head of Laboratory Dr. Yuriy V. Sheludko

ysheludko@ukr.net

Agrobacterium-Mediated Transient Expression

- Short production time
- Eukaryotic expression systems (plants)
- Wide variety of effective genetic vectors
- Wide range of proteins (including multimeric proteins)

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Agrobacterium-Mediated Transient Expression: Optimal Host Selection

Agroinjection

COMMUNICATION TO THE EDITOR

BIOTECHNOLOGY AND BIOENGINEERING

Comparison of Several *Nicotiana* Species as Hosts for High-Scale *Agrobacterium*-Mediated Transient Expression

Y.V. Sheludko, Y.R. Sindarovska, I.M. Gerasymenko, M.A. Bannikova, N.V. Kuchuk
Institute of Cell Biology and Genetic Engineering, Zabolotnogo Str. 148, Kyiv 03143, Ukraine; telephone: +380 44 5267104; fax +380 44 5221786; e-mail: ysheludko@fromru.com; sheludko@iicb.kiev.ua

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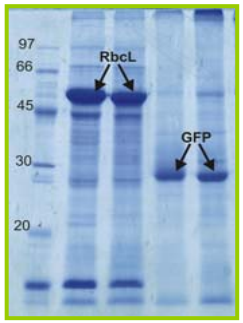
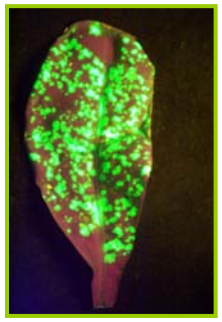
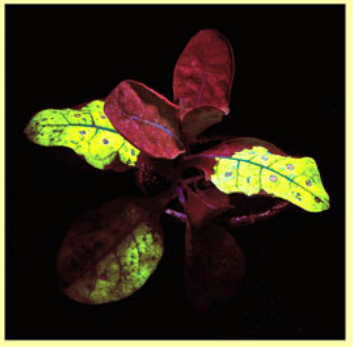
Published online 18 September 2006 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/bit.21075

GFP up to ~ 50 % TSP







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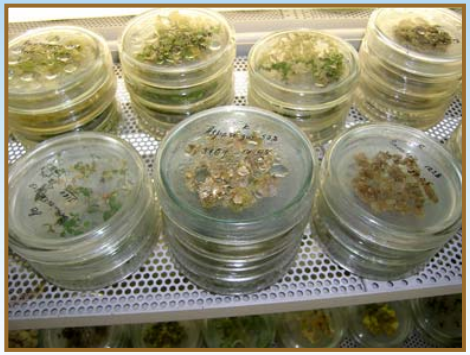
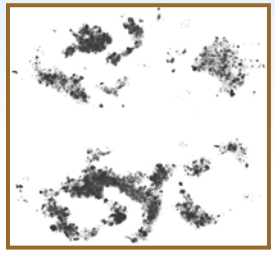
Agrobacterium-Mediated Transient Expression: Optimal Host Selection (Plant Cell Cultures)





~ 300 Species Tested

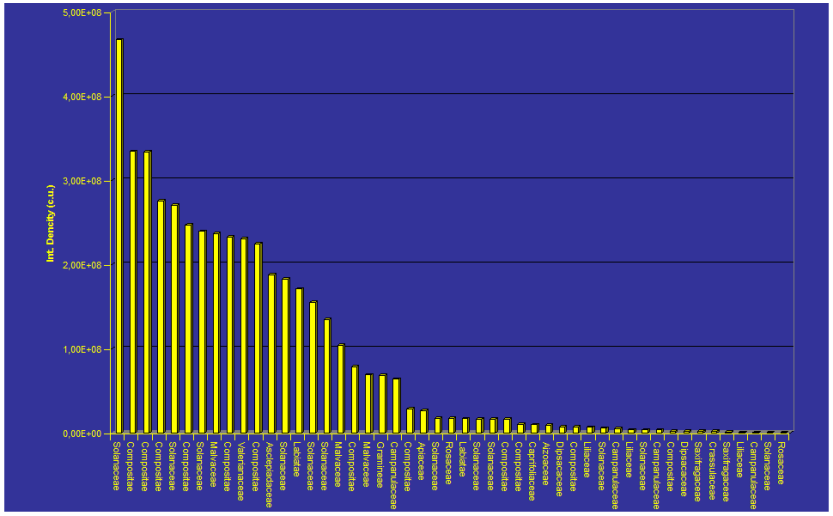
~ 20 % Exhibited Detectable Activity

The Most Activity:

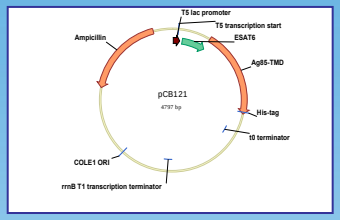
-Solanaceae

-Compositae

-Malvaceae



Computer analysis of GUS expression

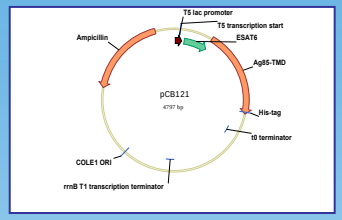


Molecular Design of Highly Effective Plant Expression and Selection Systems

Application of Optimal Regulatory and Transport Sequences

Development of Multiplex PCR Assays

Application of Hybrid Genes



Application of Optimal Regulatory and Transport Sequences: Collection of Regulatory Elements

Expression in Seeds

Promoters of A. thalianam and B. napus

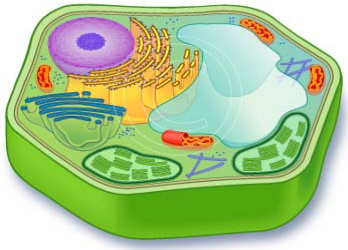
Expression in Meristems

A. thaliana promoters

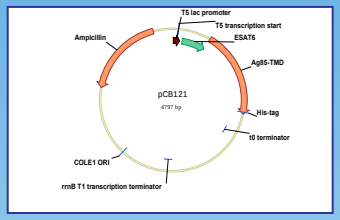
Cold-inducible Expression

A. thaliana promoters

Chloroplast Accumulation

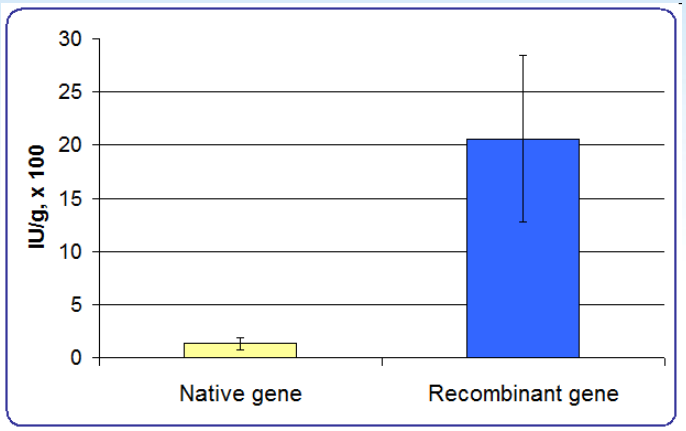


Transit Peptides of *N. tabacum and Spinacia oleracea*

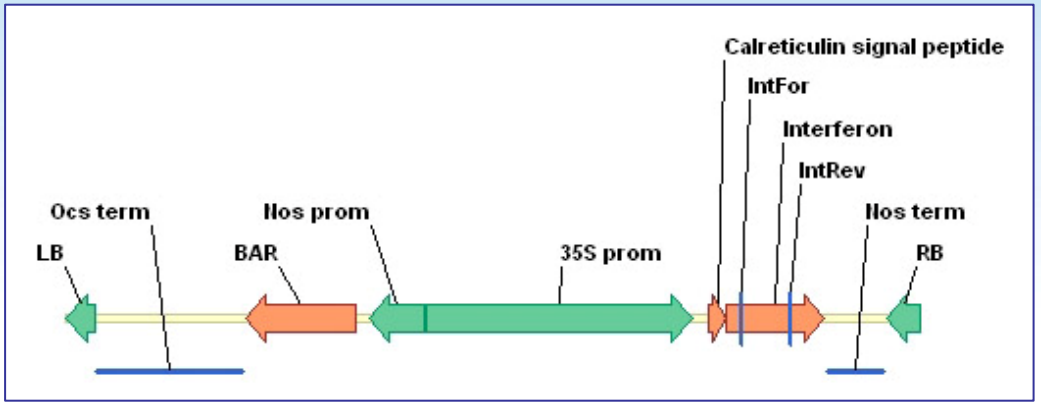


Application of Optimal Regulatory and Transport Sequences

Vectors for interferon expression with native and plant signal peptide in combination with kanamycin or PPT resistance



Activity of interferon in leaf extracts of *N. excelsior* transiently expressing native or recombinant (fused to *N. plumbaginifolia* calreticulin signal peptide) human interferon α2b gene.



ISSN 0095-4527, Cytology and Genetics, 2010, Vol. 44, No. 5, pp. 313–316. © Allerton Press, Inc., 2010.

Production of Human Interferon ALPHA 2b in Plants of *Nicotiana excelsior* by *Agrobacterium*-Mediated Transient Expression[1]

Y. R. Sindarovska[a], I. M. Gerasymenko[a], Y. V. Sheludko[a], Z. M. Olevinskaya[b], N. Y. Spivak[b], and N. V. Kuchuk[a]

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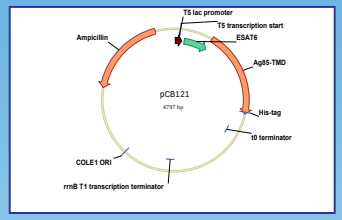
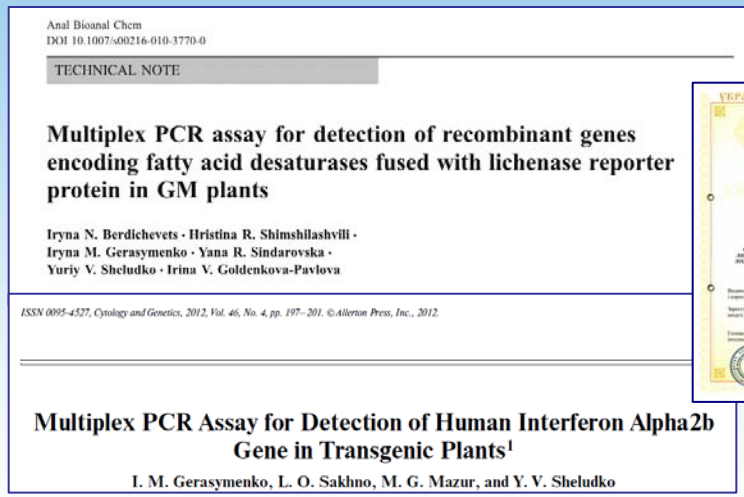


Multiplex PCR Assays



Anal Bioanal Chem
DOI 10.1007/s00216-010-3770-0

TECHNICAL NOTE

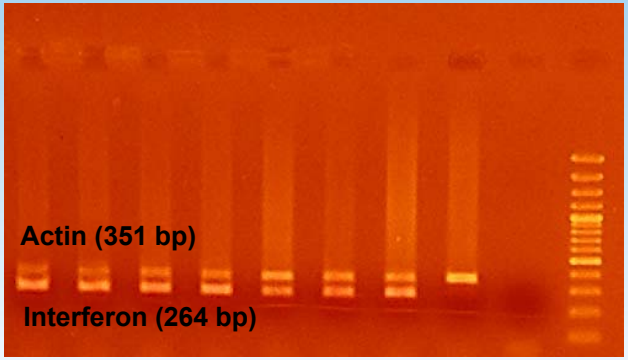
Multiplex PCR assay for detection of recombinant genes encoding fatty acid desaturases fused with lichenase reporter protein in GM plants

Iryna N. Berdichevets · Hristina R. Shimshilashvili · Iryna M. Gerasymenko · Yana R. Sindarovska · Yuriy V. Sheludko · Irina V. Goldenkova-Pavlova

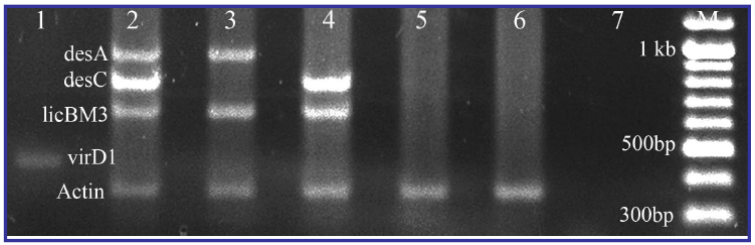
ISSN 0095-4527, Cytology and Genetics, 2012, Vol. 46, No. 4, pp. 197–201. © Allerton Press, Inc., 2012.

Multiplex PCR Assay for Detection of Human Interferon Alpha2b Gene in Transgenic Plants[1]

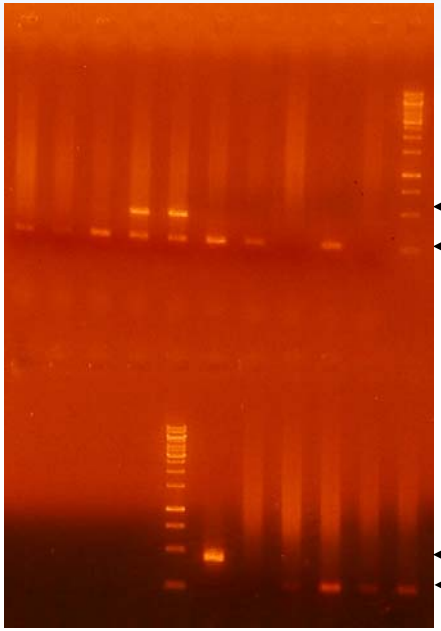
I. M. Gerasymenko, L. O. Sakhno, M. G. Mazur, and Y. V. Sheludko



Multiplex PCR analysis of human interferon α2b and house-keeping actin gene (top)



Multiplex PCR analysis of GM tobacco for detection of recombinant *lic*BM3, *des*A and *des*C genes, house-keeping actin gene and *vir*D1 gene of *Agrobacterium tumefaciens*.



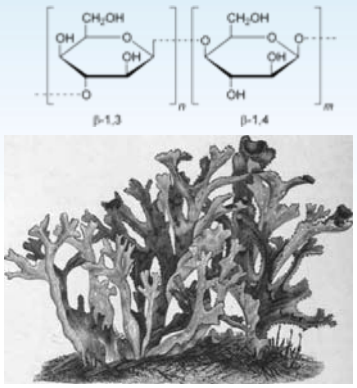
*vir*D1 (432 bp)
Interferon (264 bp)

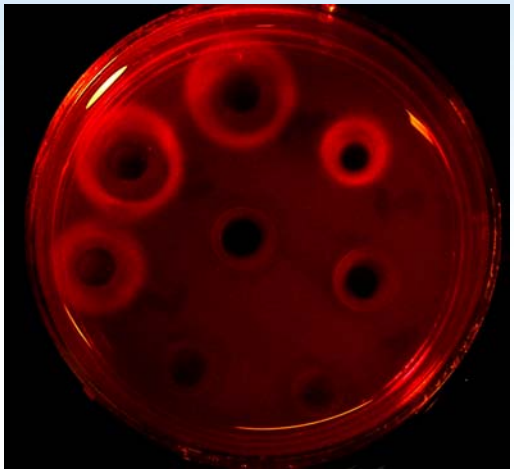
and interferon a2b and *vir*D1 (bottom)

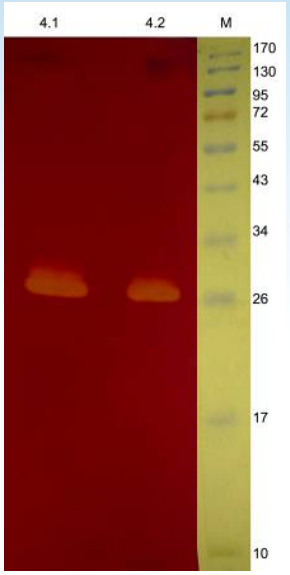
*vir*D1 (432 bp)
Interferon (264 bp)

Application of Hybrid Genes

1,3-1,4 ß-Glucanase (Lichenase): Glycoside Hydrolase from *Clostridium thermocellum*







Lichenase As a Reporter and Carrier:

- ✓High Detection Specificity (Activity max at 70°C);
- ✓Small Size and High Activity in Hybrid Genes
- ✓Qualitative and Quantitative Activity Analysis;
- ✓Zymogramme vs. Western-Blotting

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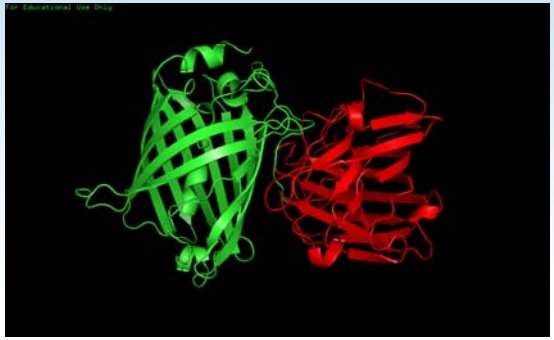
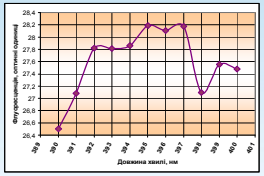
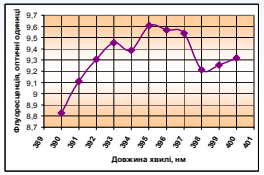
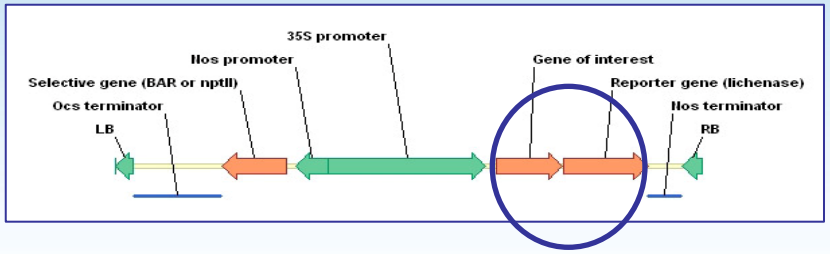
Application of Hybrid Genes

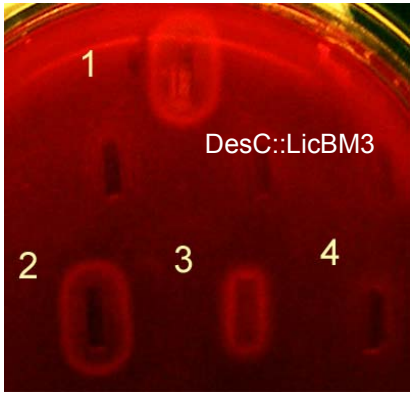
GFP::LicBM3 - Bifunctional Reporter for Expression in Plants

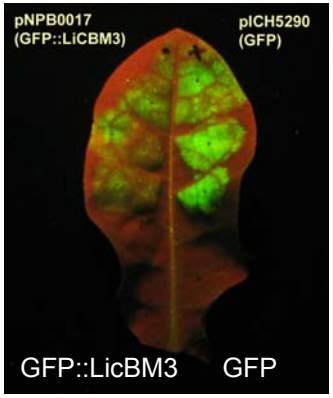


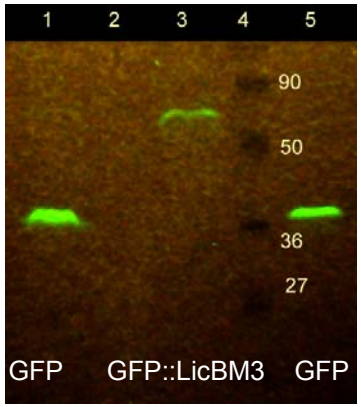


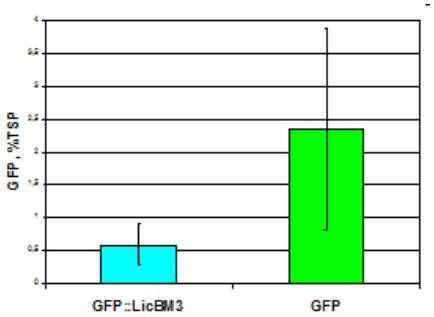












Analysis of
GFP::LicBM3
Functional Activity

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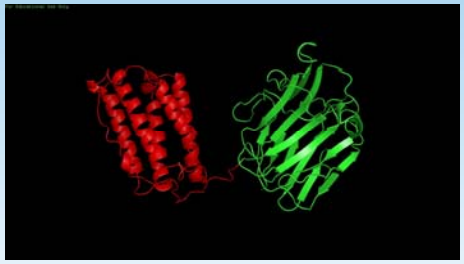
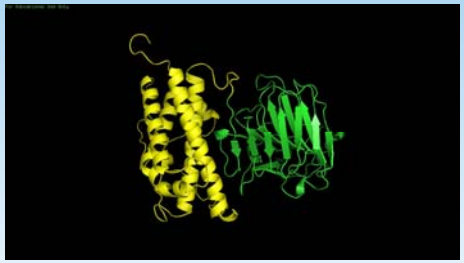
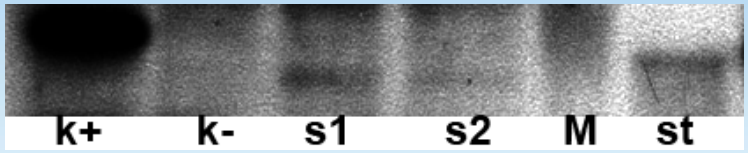
Application of Hybrid Genes

hGH::LicBM3 and Ifn::LicBM3 – Hybrid Pharm. Protein Genes for Expression in Plants

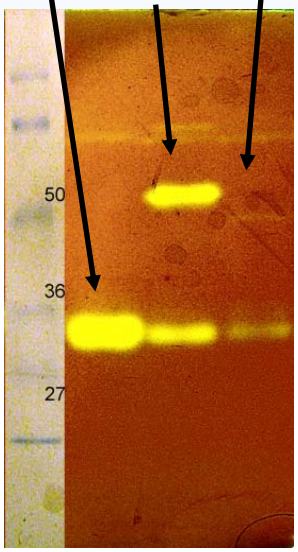




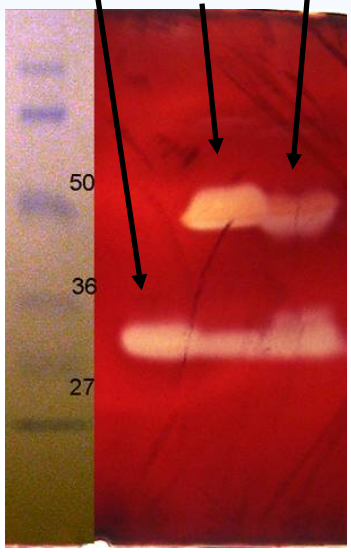


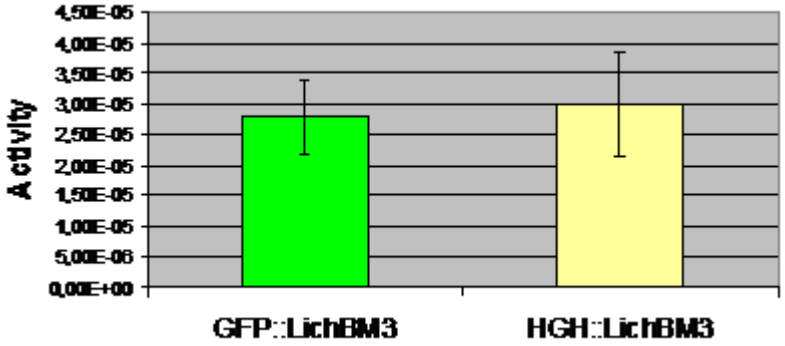
hGH

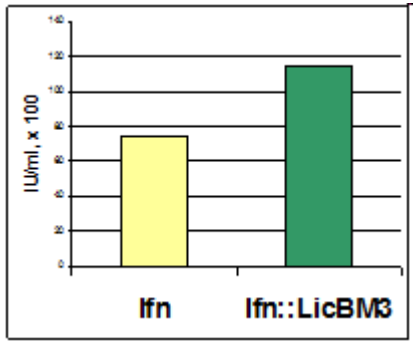
GFP: Inf: LicB :LicB :LicB



GFP: hGH: LicB :LicB :LicB







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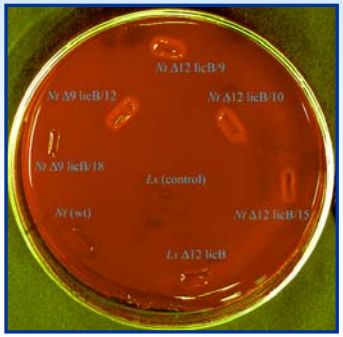
Expression of Cyanobacteria Desaturase::Lichenase Hybrid Genes in Plants



Detection of Hybrid Gene Expression in Transgenic Plants by Lichenase Activity Test.

ISSN 1993–6842. Biopolymers and Cell. 2012. Vol. 28. N 6. P. 449–455 (Translated from Russian) doi 10.7124/bc.000135

UDC 575.827:604.6:582.683.2

Creation of glyphosate-resistant *Brassica napus* L. plants expressing DesC desaturase of cyanobacterium *Synechococcus vulcanus*

L. O. Sakhno[1], I. M. Gerasymenko[1], I. K. Komarnitsskii[1], Y. V. Sheludko[1], I. V. Goldenkova-Pavlova[2]

Вестник ВОГиС. 2010. Том 14. № 1 127

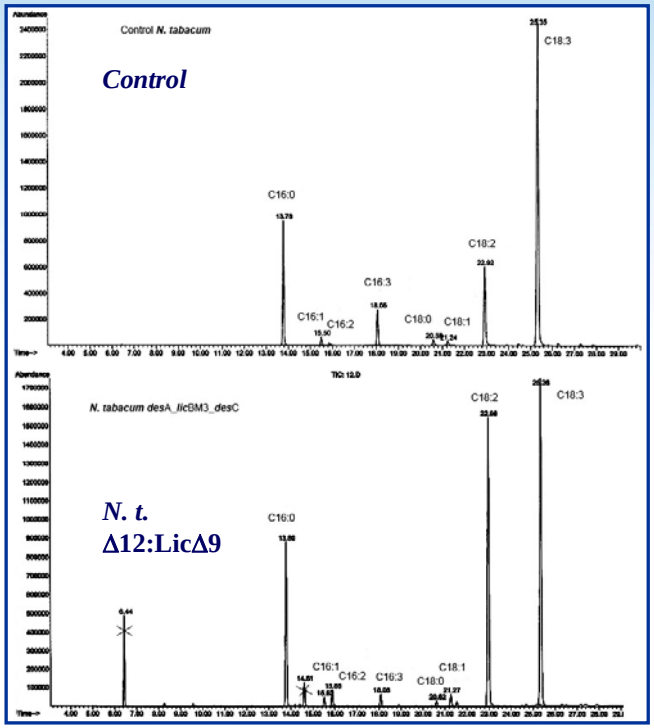
RAISE OF PLANTS POSSESSING GENES FOR ACYL-LIPID DESATURASES FROM THE CYANOBACTERIA

I.M. Gerasymenko[1], L.O. Sakhno[1], I.S. Golovach[1], O.M. Kishchenko[1], Y.R. Sindarovska[1], C.R. Shimshilashvili[2], Y.V. Sheludko[1], I.V. Goldenkova-Pavlova[2]

***N. t.* Δ9:Lic**

***N. t.* Δ12:Lic**

***N. t.* Δ12:Lic Δ9**



Fatty Acid Ratio in Control and Transgenic Plants.

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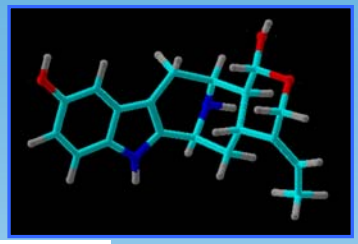
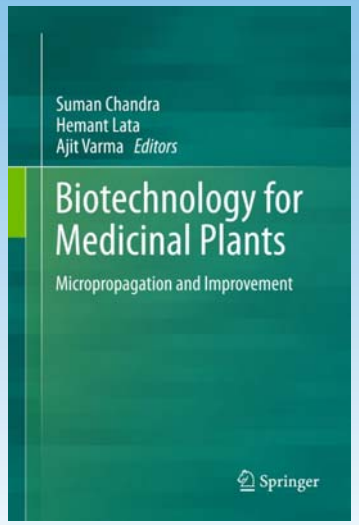


Alkaloid biosynthesis in *Rauvolfia Serpentina* Hairy Root Cultures

(in cooperation with Institute of Pharmacy, Mainz)

New Alkaloids from R.serpentina hairy roots



Chapter 10
Biosynthetic Potential of Hairy Roots for Production of New Natural Products

Yuriy Sheludko and Iryna Gerasymenko

1006 *J. Nat. Prod.* **2002**, *65*, 1006−1010

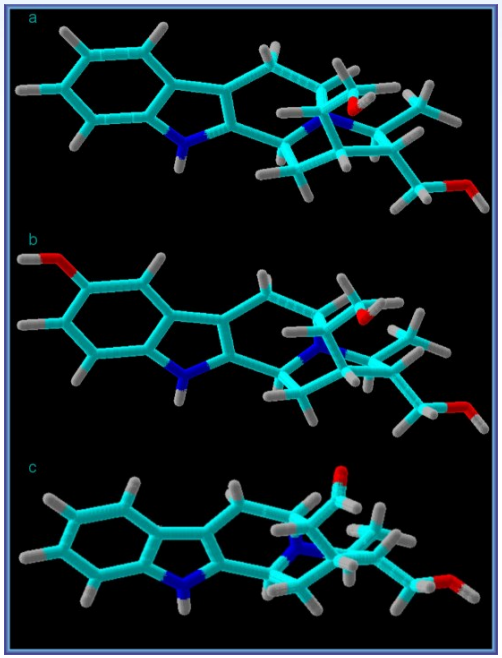
New Alkaloids of the Sarpagine Group from *Rauvolfia serpentina* Hairy Root Culture[⊥]

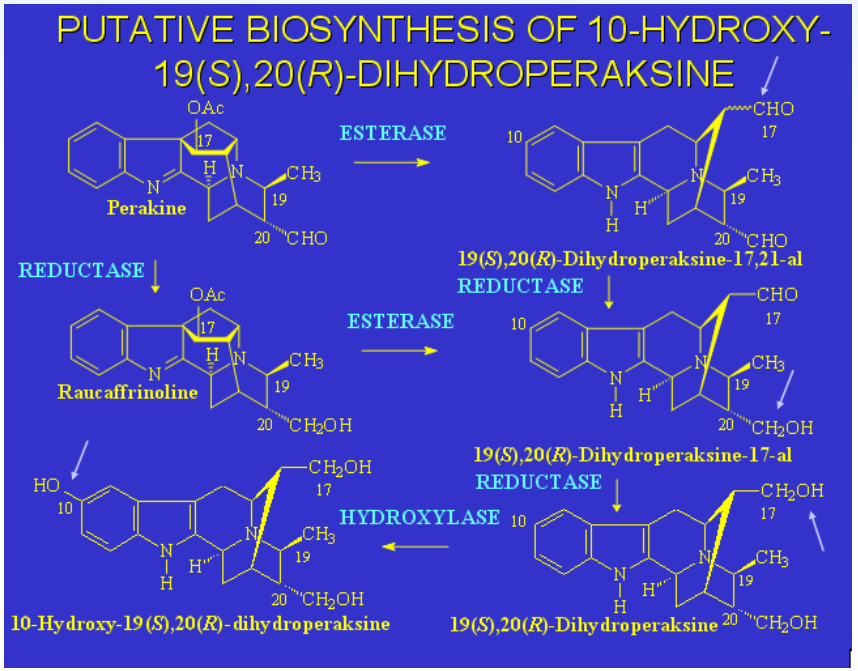
Yuri Sheludko,[†,‡] Irina Gerasimenko,[†] Heinz Kolshorn,[§] and Joachim Stöckigt*[,†]

Plant Mol Biol (2008) 67:455–467
DOI 10.1007/s11103-008-9331-7

Purification, cloning, functional expression and characterization of perakine reductase: the first example from the AKR enzyme family, extending the alkaloidal network of the plant *Rauvolfia*

Lianli Sun · Martin Ruppert · Yuri Sheludko · Heribert Warzecha · Yu Zhao · Joachim Stöckigt





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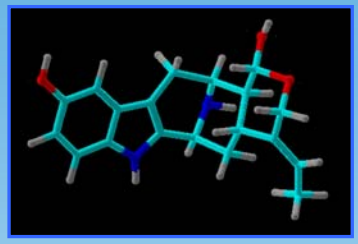
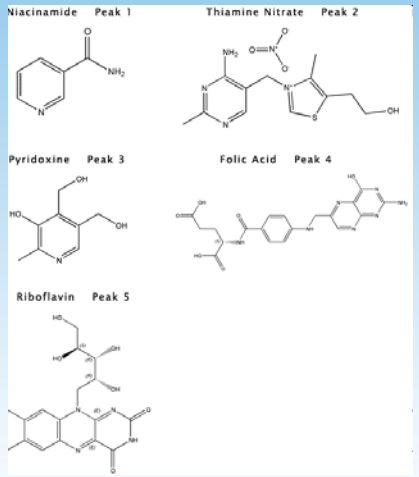
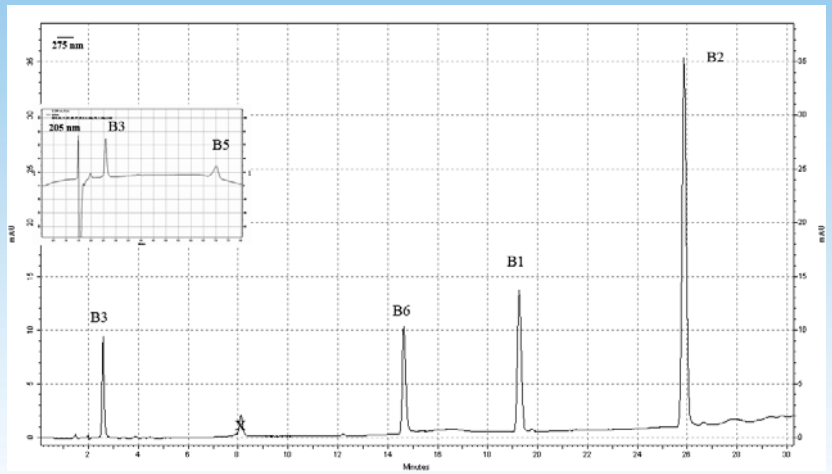
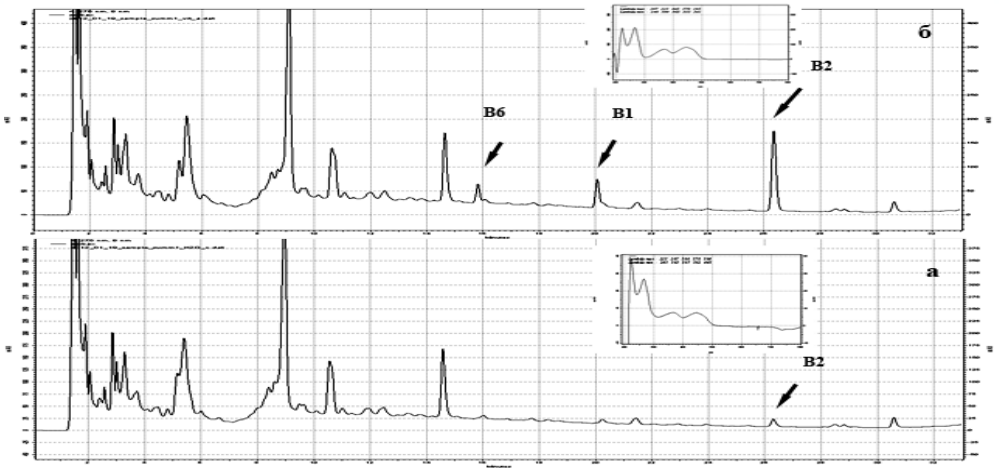


Simultaneous HPLC Detection of Water Soluble Vitamins in Biomass





B2 content in *Coriolus versicolor* extracts 12,7±1,1 mg/kg RW, B6 and B1 content < 4-5 mg/kg RW



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