



# Plant enzymes for industrial application

Why plant enzymes?

Plants produce diverse sets of *unique* chemical compounds.

To synthesise these, plants use enzymes that are not present in any other organism.

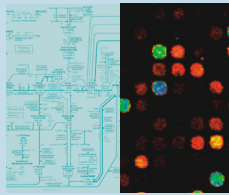
These enzymes can be deployed for diverse industrial applications.

Plant Kingdom

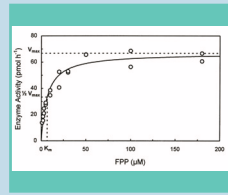
Source identification



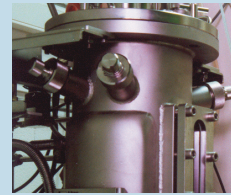
Gene identification



Functional analysis



Industrial manufacturing



Industry

Flavour

Esters, terpenes, norisoprenoids

Neutraceuticals

Flavonoids, amino acids, fructans, carotenoids

Pharma

Glycosylation, botanicals, artemisinin, antibodies, vaccines

Agrochemicals

Fungicides, toxins, insecticides, repellents

Track record: patent applications and high quality peer reviewed journals

Flavour

Aharoni et al., (2000) Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. **Plant Cell** 12(5): 647-661  
Aharoni et al., (2004) Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. **Plant Cell** 16(11): 3110-3131  
Beekwilder et al., (2004) Functional characterization of enzymes forming volatile esters from strawberry and banana. **Plant Physiol.** 135(4): 1865-1878  
deKraker et al., (2002) Biosynthesis of costunolide, dihydrocostunolide, and leucodiolin. Demonstration of cytochrome P450-catalyzed formation of the lactone ring present in sesquiterpene lactones of chichory. **Plant Physiol.** 129(1): 257-268  
deKraker et al., (2003) Hydroxylation of sesquiterpenes by enzymes from chichory (*Cichorium intybus* L.) roots. **Tetrahedron** 59(3): 409-418  
El-Tamer et al., (2003) The influence of monoterpene synthase transformation on the odour of tobacco. **J. Biotechnology** 106(1): 15-21  
Lucker et al., (2002) Monoterpene biosynthesis in lemon (*Citrus limon*) - cDNA isolation and functional analysis of four monoterpene synthases. **Eur. J. Biochem.** 269(13): 3160-3171  
Lucker et al., (2004) Metabolic engineering of monoterpene biosynthesis: two-step production of (+)-trans-isopiperitone by tobacco. **Plant J.** 39(1): 135-145  
A number of patent applications have been filed relating to these publications.

Neutraceuticals

Bovy et al., (2002) High-flavonol tomatoes resulting from the heterologous expression of the maize transcription factor genes LC and C1. **Plant Cell** 14(10): 2509-2526  
Femia et al., (2003) Effect of diets fortified with tomatoes or onions with variable quercetin glycoside content on azoxymethane-induced aberrant crypt foci in the colon of rats. **Eur. J. Nutr.** 42(6): 346-352  
Helsper et al., (2002) Quadrupole time-of-flight mass spectrometry: A method to study the actual expression of allergen isoforms identified by PCR cloning. **J. Allergy Clin. Immun.** 110(1): 131-138  
Muir et al., (2001) Overexpression of petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. **Nature Biotechnol.** 19(5): 470-474  
Sevener et al., (1998) High level fructan accumulation in a transgenic sugar beet. **Nature Biotechnol.** 16(9): 843-846  
Sevener et al., (2002) Increased production of nutriment by genetically engineered crops. **J. Am. Coll. Nutr.** 21(3): 199S-204S  
van der Meer et al., (2001) Plant-based raw material: improved food quality for better nutrition via plant genomics. **Curr. Op. Biotechnol.** 2(5): 488-492  
Van der Meer et al., (1998) Cloning of the fructan biosynthesis pathway of Jerusalem artichoke. **Plant J.** 15(4): 489-500  
Verhoeven et al., (2002) Increasing antioxidant levels in tomatoes through modification of the flavonoid biosynthetic pathway. **J. Exp. Bot.** 53(377): 2099-2106  
A number of patent applications have been filed relating to these publications.

Pharma

Bakker et al., (2001) Galactose-extended glycans of antibodies produced by transgenic plants. **Proc. Natl. Acad. Sci. U S A.** 98(5): 2899-2904  
Bakker et al., (2001) Plant members of the 1->3/4-fucosyltransferase gene family encode an 1->4-fucosyltransferase, potentially involved in Lewis(x) biosynthesis, and two core 1->3-fucosyltransferases. **FEBS-lett.** 507(3): 307-312  
Bakker et al., (1999) An Arabidopsis thaliana cDNA complements the N-acetylglucosaminyl transferase deficiency of CHO Lec1 cells. **Biochem. Biophys. Res. Comm.** 261(3): 829-832  
Beekwilder et al., (1999) A phagemid vector using the E. coli phage shock promoter facilitates phage display of toxic proteins. **Gene** 228: 23-31  
Bino et al., (2004) Potential of metabolomics as a functional genomics tool. **Trends Plant Sci.** 9(9):418-25.  
Bouwmeester et al., (1999) Amorpha-4,11-diene synthase catalyses the first probable step in artemisinin biosynthesis. **Phytochem.** 52(5): 843-854  
Bouwmeester et al., (2002) Isolation and characterization of two germacrene A synthase cDNA clones from chichory. **Plant Physiol.** 129(1): 134-144  
deKraker et al., (2001) Germacrenes from fresh costus roots. **Phytochem.** 58(3): 481-487  
Ceci et al., (2003) Selection by phage display of a mustard chymotrypsin inhibitor toxic to pea aphid. **Plant J.** 33: 557-566  
Gales et al., (2003) Structural characterization of thyroglobulin type-1 domains of equistatin. **FEBS Lett.** 539:120-124  
Lauterslager et al., (2001) Oral immunisation of naive and primed animals with transgenic potato tubers expressing LT-B. **Vaccine** 19(17-19): 2749-2755  
Outchkourov et al., (2002) Optimization of the expression of equistatin in *Pichia pastoris*. **Prot. Expr. Purif.** 24:18-24  
Rogel et al., (2000) Expression, purification and characterisation of equistatin in *Pichia pastoris*. **Prot. Express. Purif.** 19: 329-334  
Schmidt et al., (1999) Mechanisms of the biosynthesis of sesquiterpene enantiomers (+) and (-)-germacren D in *Solidago canadensis*. **Chirality** 11(5-6): 353-362  
A number of patent applications have been filed relating to these publications.

Agrochemicals

Aharoni et al., (2003) Terpenoid metabolism in wild-type and transgenic Arabidopsis plants. **Plant Cell** 15(12): 2866-2884  
Balkema-Boostrma et al., (2003) Role of cucurbitacin C in resistance to spider mite (*Tetranychus urticae*) in cucumber (*Cucumis sativus* L.). **J. Chem. Ecol.** 29(1): 225-235  
Bouwmeester et al., (2004) Biosynthesis of the monoterpenes limonene and carvone in the fruit of caraway - I. Demonstration of enzyme activities and their changes with development. **Plant Physiol.** 117(3): 901-912  
Duetz et al., (2003) Biotransformation of limonene by bacteria, fungi, yeasts and plants. **Appl. Microbiol. Biotechnol.** 61: 269-277  
Estebanez-Perpina E et al., (2001) Crystal structure of a novel mid-gut procarboxypeptidase from the cotton pest *Helicoverpa armigera*. **J. Mol. Biol.** 313(3): 629-638  
Jongsma et al., (1995) Adaptation of *Spodoptera exigua* larvae to plant proteinase inhibitors by induction of gut proteinase activity insensitive to inhibition. **Proc. Natl. Acad. Sci. U S A.** 92(17): 8041-5.  
Lucker et al., (2001) Expression of Clarkia  $\beta$ -linalool synthase in transgenic petunia plants results in the accumulation of  $\beta$ -linalyl-beta-D-glucopyranoside. **Plant J.** 27(4): 315-324  
Volpicella et al., (2003) Properties of purified gut trypsin from *Helicoverpa zea*, adapted to proteinase inhibitors. **Eur. J. Biochem.** 270(1): 10-19  
A number of patent applications have been filed relating to these publications.