Publikationen - Natur- und Wirkstoffchemie

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Zabel, S.; Brandt, W.; Porzel, A.; Athmer, B.; Kortbeek, R. W. J.; Bleeker, P. M.; Tissier, A.; Two novel 7-epi-zingiberene derivatives with biological activity from Solanum habrochaites are

produced by a single cytochrome P450 monooxygenase *bioRxiv* (2020) DOI: 10.1101/2020.04.21.052571

Abstract
RIS
BibTeX

Secretions from glandular trichomes potentially protect the plant against a variety of aggressors. In the tomato genus, wild species constitute a rich source of chemical diversity produced at the leaf surface by glandular trichomes. Previously, 7-epi-zingiberene produced in several accessions of Solanum habrochaites was found to confer resistance to whiteflies (Bemisia tabaci) and other insect pests. Here, we identify two derivatives of 7-epi-zingiberene from S. habrochaites that had not been reported as yet. We identified them as 9-hydroxy-zingiberene and 9-hydroxy-10,11-epoxyzingiberene. Using a combination of genetics and transcriptomics we identified a single cytochrome P450 oxygenase, ShCYP71D184 that carries out two successive oxidations to generate the two sesquiterpenoids. Bioactivity assays showed that only 9-hydroxy-10,11-epoxyzingiberene exhibits substantial toxicity against B. tabaci. In addition, both 9-hydroxy-zingiberene and 9-hydroxy-10,11-epoxyzingiberene display substantial growth inhibitory activities against a range of microorganisms, including Bacillus subtilis, Phytophtora infestans and Botrytis cinerea. Our work shows that trichome secretions from wild tomato species can provide protection against a wide variety of organisms. In addition, the availability of the genes encoding the enzymes for the pathway of 7-epi-zingiberene derivatives makes it possible to introduce this trait in cultivated tomato by precision breeding.

Püllmann, P.; Knorrscheidt, A.; Münch, J.; Palme, P. R.; Hoehenwarter, W.; Marillonnet, S.; Alcalde, M.; Westermann, B.; Weissenborn, M. J.; A modular two yeast species secretion system for the production and preparative application of fungal peroxygenases *bioRxiv* (2020) DOI: 10.1101/2020.07.22.216432

Abstract
RIS
BibTeX

Fungal unspecific peroxygenases (UPOs) are biocatalysts of outstanding interest. Providing access to novel UPOs using a modular secretion system was the central goal of this work. UPOs represent an enzyme class, catalysing versatile oxyfunctionalisation reactions on a broad substrate scope. They are occurring as secreted, glycosylated proteins bearing a haem-thiolate active site and solely rely on hydrogen peroxide as the oxygen source. Fungal peroxygenases are widespread throughout the fungal kingdom and hence a huge variety of UPO gene sequences is available. However, the heterologous production of UPOs in a fast-growing organism suitable for high throughput screening has only succeeded once—enabled by an intensive directed evolution campaign. Here, we developed and applied a modular Golden Gate-based secretion system, allowing the first yeast production of four active UPOs, their one-step purification and application in an enantioselective conversion on a preparative scale. The Golden Gate setup was designed to be broadly applicable and consists of the three module types: i) a signal peptide panel guiding secretion, ii) UPO genes, and iii) protein tags for purification and split-GFP detection. We show that optimal signal peptides could be selected for successful UPO secretion by combinatorial testing of 17 signal peptides for each UPO gene. The modular episomal system is suitable for use in Saccharomyces cerevisiae and was transferred to episomal

and chromosomally integrated expression cassettes in Pichia pastoris. Shake flask productions in Pichia pastoris yielded up to 24 mg/L secreted UPO enzyme, which was employed for the preparative scale conversion of a phenethylamine derivative reaching 98.6 % ee. Our results demonstrate a rapid workflow from putative UPO gene to preparative scale enantioselective biotransformations.

Stark, P.; Zab, C.; Porzel, A.; Franke, K.; Rizzo, P.; Wessjohann, L. A.; PSYCHE - a valuable experiment in plant NMR-metabolomics *RADAR* (2020) DOI: 10.22000/338

Abstract
Internet
RIS
BibTeX

Dataset: NMR raw dataInstrument: Agilent VNMRS 600 NMR spectrometer

Steinbeck, C.; Koepler, O.; Bach, F.; Herres-Pawlis, S.; Jung, N.; Liermann, J. C.; Neumann, S.; Razum, M.; Baldauf, C.; Biedermann, F.; Bocklitz, T. W.; Boehm, F.; Broda, F.; Czodrowski, P.; Engel, T.; Hicks, M. G.; Kast, S. M.; Kettner, C.; Koch, W.; Lanza, G.; Link, A.; Mata, R. A.; Nagel, W. E.; Porzel, A.; Schlörer, N.; Schulze, T.; Weinig, H.-G.; Wenzel, W.; Wessjohann, L. A.; Wulle, S.; NFDI4Chem - Towards a National Research Data Infrastructure for Chemistry in Germany *Res. Ideas Outcomes* **6**, e55852, (2020) DOI: 10.3897/rio.6.e55852

Abstract
RIS
BibTeX

The vision of NFDI4Chem is the digitalisation of all key steps in chemical research to support scientists in their efforts to collect, store, process, analyse, disclose and re-use research data. Measures to promote Open Science and Research Data Management (RDM) in agreement with the FAIR data principles are fundamental aims of NFDI4Chem to serve the chemistry community with a holistic concept for access to research data. To this end, the overarching objective is the development and maintenance of a national research data infrastructure for the research domain of chemistry in Germany, and to enable innovative and easy to use services and novel scientific approaches based on re-use of research data. NFDI4Chem intends to represent all disciplines of chemistry in academia. We aim to collaborate closely with thematically related consortia. In the initial phase, NFDI4Chem focuses on data related to molecules and reactions including data for their experimental and theoretical characterisation.This overarching goal is achieved by working towards a number of key objectives:Key Objective 1: Establish a virtual environment of federated repositories for storing, disclosing, searching and re-using research data across distributed data sources. Connect existing data repositories and, based on a requirements analysis, establish domain-specific research data repositories for the national research community, and link them to international repositories.Key Objective 2: Initiate international community processes to establish minimum information (MI) standards for data and machine-readable metadata as well as open data standards in key areas of chemistry. Identify and

recommend open data standards in key areas of chemistry, in order to support the FAIR principles for research data. Finally, develop standards, if there is a lack.Key Objective 3: Foster cultural and digital change towards Smart Laboratory Environments by promoting the use of digital tools in all stages of research and promote subsequent Research Data Management (RDM) at all levels of academia, beginning in undergraduate studies curricula.Key Objective 4: Engage with the chemistry community in Germany through a wide range of measures to create awareness for and foster the adoption of FAIR data management. Initiate processes to integrate RDM and data science into curricula. Offer a wide range of training opportunities for researchers.Key Objective 5: Explore synergies with other consortia and promote cross-cutting development within the NFDI.Key Objective 6: Provide a legally reliable framework of policies and guidelines for FAIR and open RDM.

Stefan, K.; Wen Leck, L. Y.; Namasivayam, V.; Bascuñana, P.; Huang, M. L.-H.; Riss, P. J.; Pahnke, J.; Jansson, P. J.; Stefan, S. M.; Vesicular ATP-binding cassette transporters in human disease: relevant aspects of their organization for future drug development *Future Drug Discovery* **4**, FDD51, (2020) DOI: 10.4155/fdd-2020-0025

RIS
BibTeX

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Stark, P.; Zab, C.; Porzel, A.; Franke, K.; Rizzo, P.; Wessjohann, L. A.; PSYCHE—A Valuable Experiment in Plant NMR-Metabolomics *Molecules* **25**, 5125, (2020) DOI: 10.3390/molecules25215125

Abstract
Internet
RIS
BibTeX

1H-NMR is a very reproducible spectroscopic method and, therefore, a powerful tool for the metabolomic analysis of biological samples. However, due to the high complexity of natural samples, such as plant extracts, the evaluation of spectra is difficult because of signal overlap. The new NMR “Pure Shift” methods improve spectral resolution by suppressing homonuclear coupling and turning multiplets into singlets. The PSYCHE (Pure Shift yielded by Chirp excitation) and the Zangger–Sterk pulse sequence were tested. The parameters of the more suitable PSYCHE experiment were optimized, and the extracts of 21 Hypericum species were measured. Different evaluation criteria were used to compare the suitability of the PSYCHE experiment with conventional 1H-NMR. The relationship between the integral of a signal and the related bin value established by linear regression demonstrates an equal representation of the integrals in binned PSYCHE spectra compared to conventional 1H-NMR. Using multivariate data analysis based on both techniques reveals comparable results. The obtained data demonstrate that Pure Shift spectra can support the evaluation of conventional 1H-NMR experiments.

Smolko, L.; Smolková, R.; Samoľová, E.; Morgan, I.; Saoud,

M.; Kaluđerović, G. N.; Two isostructural Co(II) flufenamato and niflumato complexes with bathocuproine: Analogues with a different cytotoxic activity *J. Inorg. Biochem.* **210**, 111160, (2020) DOI: 10.1016/j.jinorgbio.2020.111160

Abstract
Internet
RIS
BibTeX

Two novel Co(II) fenamato complexes containing bathocuproine (bcp), namely [Co(bcp)(flu)2] (1) and [Co(bcp)(nif)2] (2) (flu = flufenamato, nif = niflumato) were synthesized and characterized by elemental analysis, single-crystal X-ray structure analysis as well as absorption and fluorescence spectroscopy. Investigation of their molecular structure revealed that both complexes are isostructural and form analogous complex molecules, with a Co(II) atom hexacoordinated by two nitrogen atoms of bcp and four oxygen atoms of two chelate bonded flu (1) and nif (2) ligands in a distorted octahedral arrangement. Surprisingly, the results of cytotoxicity experiments on four cancer cell lines (HeLa, HT-29, PC-3 and MCF-7) have revealed that despite similar structure of the complexes, the nif complex exhibits significantly higher activity, being the most effective against the PC-3 cell line (IC50 (MTT) = 6.11 ± 1.95 μM). Further studies performed on PC-3 cell line have shown that the mechanism of the cytotoxic action of nif complex (2) might involve activation of autophagic processes and apoptosis, while for its flu analogue (1) apoptosis was detected.

Smolikova, G.; Shiroglazova, O.; Vinogradova, G.; Leppyanen, I.; Dinastiya, E.; Yakovleva, O.; Dolgikh, E.; Titova, G.; Frolov, A.; Medvedev, S.; Comparative analysis of the plastid conversion, photochemical activity and chlorophyll degradation in developing embryos of green-seeded and yellow-seeded pea (Pisum sativum) cultivars *Funct. Plant Biol.* **47**, 409-424, (2020) DOI: 10.1071/FP19270

Abstract
RIS
BibTeX

Developing seeds of some higher plants are photosynthetically active and contain chlorophylls (Chl), which are typically destroyed at the late stages of seed maturation. However, in some crop plant cultivars, degradation of embryonic Chl remains incomplete, and mature seeds preserve green colour, as it is known for green-seeded cultivars of pea (Pisum sativum L.). The residual Chl compromise seed quality and represent a severe challenge for farmers. Hence, comprehensive understanding of the molecular mechanisms, underlying incomplete Chl degradation is required for maintaining sustainable agriculture. Therefore, here we address dynamics of plastid conversion and photochemical activity alterations, accompanying degradation of Chl in embryos of yellow- and green-seeded cultivars Frisson and Rondo respectively. The yellow-seeded cultivar demonstrated higher rate of Chl degradation at later maturation stage, accompanied with termination of photochemical activity, seed dehydration and conversion of green plastids into amyloplasts. In agreement with this, expression of genes encoding enzymes of Chl degradation was lower in the green seeded cultivar, with the major differences in the levels of Chl b reductase (NYC1)

and pheophytinase (PPH) transcripts. Thus, the difference between yellow and green seeds can be attributed to incomplete Chl degradation in the latter at the end of maturation period.

Smolikova, G.; Gorbach, D.; Lukasheva, E.; Mavropolo-Stolyarenko, G.; Bilova, T.; Soboleva, A.; Tsarev, A.; Romanovskaya, E.; Podolskaya, E.; Zhukov, V. A.; Tikhonovich, I.; Medvedev, S.; Hoehenwarter, W.; Frolov, A.; Bringing new methods to the seed proteomics platform: Challenges and perspectives *Int. J. Mol. Sci.* **21**, 9162, (2020) DOI: 10.3390/ijms21239162

Abstract
Internet
RIS
BibTeX

For centuries, crop plants have represented the basis of the daily human diet. Among them, cereals and legumes, accumulating oils, proteins, and carbohydrates in their seeds, distinctly dominate modern agriculture, thus play an essential role in food industry and fuel production. Therefore, seeds of crop plants are intensively studied by food chemists, biologists, biochemists, and nutritional physiologists. Accordingly, seed development and germination as well as age- and stress-related alterations in seed vigor, longevity, nutritional value, and safety can be addressed by a broad panel of analytical, biochemical, and physiological methods. Currently, functional genomics is one of the most powerful tools, giving direct access to characteristic metabolic changes accompanying plant development, senescence, and response to biotic or abiotic stress. Among individual post-genomic methodological platforms, proteomics represents one of the most effective ones, giving access to cellular metabolism at the level of proteins. During the recent decades, multiple methodological advances were introduced in different branches of life science, although only some of them were established in seed proteomics so far. Therefore, here we discuss main methodological approaches already employed in seed proteomics, as well as those still waiting for implementation in this field of plant research, with a special emphasis on sample preparation, data acquisition, processing, and post-processing. Thereby, the overall goal of this review is to bring new methodologies emerging in different areas of proteomics research (clinical, food, ecological, microbial, and plant proteomics) to the broad society of seed biologists.

Sheludko, Y. V.; Volk, J.; Brandt, W.; Warzecha, H.; Expanding the diversity of plant monoterpenoid indole alkaloids employing human cytochrome P450 3A4 *ChemBioChem* **21**, 1976-1980, (2020) DOI: 10.1002/cbic.202000020

Abstract
RIS
BibTeX

Human drug-metabolizing cytochrome P450 monooxygenases (CYPs) have enormous substrate promiscuity; this makes them promising tools for the expansion of natural product diversity. Here, we used CYP3A4 for the targeted diversification of a plant biosynthetic route leading to monoterpenoid indole alkaloids. In silico, in vitro and in planta studies proved that CYP3A4 was able to convert the indole alkaloid vinorine into

vomilenine, the former being one of the central intermediates in the ajmaline pathway in the medicinal plant Rauvolfia serpentina (L.) Benth. ex Kurz. However, to a much larger extent, the investigated conversion yielded vinorine (19R ,20R)-epoxide, a new metabolite with an epoxide functional group that is rare for indole alkaloids. The described work represents a successful example of combinatorial biosynthesis towards an increase in biodiversity of natural metabolites. Moreover, characterisation of the products of the in vitro and in planta transformation of potential pharmaceuticals with human CYPs might be indicative of the route of their conversion in the human organism.

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