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Abstract: The scientific work of Jan-Peter Frahm and others elucidated the enormous potential bryophytes have for basic as well as applied research. In this review we focus on some results that widely open the door for the use of different bryophyte species for plant biotechnology, suggesting that "Bryotechnology" is a rapidly evolving sector of biotechnology in general.

Keywords: functional genomics, biopharmaceuticals, stress tolerance, metabolomics, International Moss Stock Center

Introduction

Over the centuries bryologists felt that their research objects were underestimated by scientists working with animals, humans, or flowering plants. One of the first such statements can be traced back to Hedwig (1793), and nothing seemed to have changed since than. On the other hand, the first description of sex chromosomes in plants, of the continuity of chromosomes during the mitotic cycle and of non-Mendelian inheritance as well as the introduction of UV-mutagenesis to genetic research are landmarks in the life sciences first achieved by researchers working on bryophytes (reviewed in Reski 1998).

In the last decades, this situation has begun to change, as more and more publications from Jan-Peter Frahm and others appeared demonstrating that bryophytes have, due to their specific position in the evolution of land plants and thus their specific life-style, an unexpected high potential for applied research with implications for the improvement of crop plants and for human health. A selection of such reports is highlighted in this review.

Secondary Metabolites

Bryophytes possess a large variety of secondary metabolites (Asakawa 1981, Zinsmeister et al. 1991) and thus provide a considerable potential for biotechnological and biopharmaceutical applications. During the last decades more than 400 novel chemical compounds were isolated from bryophytes and structurally elucidated (Asakawa 2007). Typical compounds of mosses are flavonoids, biflavonoids, terpenes and terpenoids, like di- and triterpenoids, whereas liverworts contain a large variety of lipophilic mono-, di- and sesquiterpenoids as well as aromatic compounds like bibenzyls, benzoates, cinnamates or naphtalenes (Asakawa 2007). These aromatic compounds are responsible for the characteristic scents of several liverwort species, like the carrot-like aroma of *Jungermannia odobata* and *Nardia scalaris* (Fig. 1) or the cedar-like odor of *Jungermannia cordifolia* or *Lophocolea heterophylla* (Frahm 2001).

It is widely known that many of the secondary compounds found in bryopyhtes are biologically active substances. From the evolutionary and ecological point of view these biologically active compounds are likely involved in pathogen defense and protection against microbial infections. Compared to tracheophytes, bryophytes have no mechanical protection like bark or cuticule. Furthermore most bryophytes grow on forest ground in a close connection to several biodegrading destruents. Hence, a protection against pathogens like fungi or bacteria is essential for surviving in this habitat. Antibacterial activity against gram-positive and gram-negative bacteria was reported from mosses (Basile et al. 1999, Merkuria et al. 2005, Zhu et al. 2006) and liverworts (Asakawa 2007). Aqueous bryophyte extract inhibits the growth of *Escherichia coli* as tested on plates (Frahm, personal communication). However, this antibacterial activity seems to be specific for certain bryophyte species, as the extracts of *Marchantia polymorpha*, *Porella platyphylla* and the moss *Dicranum scoparium* showed antimicrobial effects on the gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Sarcina lutea*, but no

activity against gram-negative *E. coli* (Pavletic & Stilinovic 1963, Frahm 2004).

The fungicidal activity of bryophytes (Wolters 1964, Dikshit 1982) was recently testet in *in vivo* experiments at Bonn University. Different crops growing in greenhouses, like tomatoes, wheat and green pepper were infected with the pathogens *Phytophora infestans*, *Erysiphe graminis* and *Botrytis cinerea* and subsequently treated with alcoholic extracts from different bryophytes. Depending on the concentration, all bryophyte extracts showed a species-specific antifungal activity against the plant pathogenic fungi (Frahm 2004). Furthermore, extracts from *Neckera crispa* and *Porella obtusata* showed antifeeding effects against the portuguese slug *Aarion lusitanicus* (Frahm & Kirchhoff 2002). Given that bryophyte extracts showed fungicidal and antifeedant effects, a commercial product was developed and is sold as natural pesticide (Frahm 2004). Bryophyte extract also showed effects on human pathogenic fungi although the bioactive compounds may cause allergenic effects and dermatitis in some cases (Ando & Matsuo 1984). Due to this risk of allergic reactions bryophyte extracts were not recommended for scientific medicinal use so far (Frahm 2004). Nevertheless, with regard to the impressive complexity of secondary metabolites among bryophytes their manifold application in traditional medicine is not astonishing. Many different bryophytes, e.g. *Sphagnum*, *Marchantia*, *Riccia*. *Barbula*, *Bryum*, *Octeblepharum* and *Fontinalis* are used to treat different diseases, including cardiovascular diseases, inflammation, fever, lung diseases, infections, wounds and skin diseases (Glime 2007). In China more than 30 species can be bought at the local pharmacist (Ding 1982). Among those is for example *Polytrichum commune* (Fig. 1) which is used against fever and inflammation (Ding 1982) or boiled as a tea for treating the cold (Gulabani 1974). Another species in traditional medicine is *Rhodobryum giganteum* (Fig. 1) used to treat, among other diseases, cardiovascular diseases or angina (Glime 2007).

Beside their effects on microorganisms, bryophytes seem to affect the growth of tracheophytes as well. On the one hand bryophyte extracts show inhibiting effects and on the other hand promoting effects on seedling germination depending on the species. It was shown that extract of the liverwort *Porella platyphylla* inhibits the growth of radice seedlings, whereas the extract of *Brachythecium rutabulum* promotes the growth of radice seedlings (Frahm, personal communication).
Physcomitrella patens releases a tetracyclic diterpene, namely 16α–hydroxykaurane (16α-hydroxy-*ent*-kaurane, Kaurenol, $C_{20}H_{34}O$) in axenic culture (Von Schwartzenberg et al. 2004). This diterpene is produced in great quantities and it appears as crystal-like, needle-shapes structures on the axenic agar cultures (Von Schwartzenberg et al. 2004). The function of 16α–hydroxykaurane remains unclear so far, but it is assumed to be bioactive. 16α–hydroxykaurane is known to be produced by lichen species and fungi (Dayan & Romagni 2001) and it is commonly known from *Gibberella fujikuroi*, a plant pathogenic fungus that infects rice plants and causes foolish rice seedling disease. As recently shown, 16α–hydroxykaurane is involved in spore germination in *Physcomitrella patens* and leads to a complete inhibition of spore germination when applied in high concentrations of 2-3µM (Anterola et al. 2009).

Summing up, bryophytes possess numerous chemical compounds of biotechnological and biopharmaceutical interest. Several secondary metabolites were isolated so far from different species but the mechanisms behind their activity are still widely unexplored.

Stress tolerance and stress-related genes

Mosses were among the first plants making the step from a life in water to a life on land and they successfully colonised terrestrial habitats. The major demand to pioneer these habitats was the acquisition of molecular, biochemical and physiological

Figure 1. *Rhodobryum giganteum*, *Polytrichum commune* and *Nardia scalaris*. *Rhodobryum giganteum* is used in traditional medicine to treat angina and cardiovascular diseases, *Polytrichum commune* is used against fever and inflammation. *Nardia scalaris* is known for its characteristic odor due to aromatic compounds. (Pictures by Jan-Peter Frahm)

mechanisms to cope with new adverse environmental conditions such as radiation, water deficiency, heat and cold. Even today the growth of bryophytes in specific habitats such as the margins of lakes and rivers with changing water availability requires specific adaptive programmes to ensure survival of the species. In addition, while flowering plants developed specialised tissues to transport and retain water, the bryophytes retained a simple, non-vascular morphology. Most moss tissues are only one cell layer in thickness and are in direct contact to the environment. Moreover, mosses do not possess stomata to balance assimilation and water loss and the water content of the cells is equivalent to that of their environment (poikilohydric plants). Based on these specific features of bryophytes it is evident that mosses have acquired and maintained particular adaptive mechanisms to withstand unfavourable environmental conditions.

In the past different moss model species were chosen to analyse their physiological tolerance against various abiotic stress factors and to gain insight into the underlying molecular mechanisms. A well-established species is the drought-tolerant moss *Tortula ruralis* that is able to withstand complete desiccation of its vegetative tissues (desiccation tolerant) and returns to active metabolism and growth within minutes upon rehydration (Oliver et al. 2004). *T. ruralis* was used to identify genes that show specific changes in their expression upon dehydration and rehydration (Wood et al. 1999; Oliver et al. 2004; Oliver et al. 2009). Analysis of EST data led to the hypothesis that the high degree of dehydration tolerance of *T. ruralis* is based on the constitutive expression of stress-related genes. Thus, *T. ruralis* exhibits a preparedness to face abiotic stress conditions. In addition, the molecular analysis during the dehydration process revealed that particular mRNAs are sequestered and stabilised in mRNA/protein complexes that facilitate the rapid translation of rehydrins after the uptake of water (Wood et al. 2000).

In recent years *P. patens* emerged as another model system to study mechanisms of abiotic stress adaptation, to identify stress-related genes and to characterise them functionally by reverse genetics approaches. *P. patens* cannot withstand complete water loss, but tolerates water loss of up to 92% and is classified to be dehydration tolerant. Moreover, *P. patens* shows high degree of tolerance to other abiotic stresses including salt and osmotic stress (Frank et al. 2005). The phylogenetic position of *P. patens* in land plant evolution, the availability of its genome sequence and its amenability to molecular biology approaches allows for the functional analysis of stress-related genes. One reason for the high stress tolerance of *P. patens* compared to seed plants could be the retention of stress-related genes that may have been lost during the evolution of seed plants. One example emphasising such a scenario is the presence of P-type Na^+-ATPases homologous to fungal ENA-type Na^+-ATPases that are absent in flowering plants (Benito & Rodriguez-Navarro 2003; Fraile-Escanciano et al. 2009). In consequence, flowering plants are not able to maintain low Na^+ and high K^+ concentrations at high external Na^+, whereas *P. patens* is able to adjust cytosolic Na^+ concentrations by active transport of Na^+ ions over the plasma membrane. Furthermore, *P. patens* can serve as a model system to analyse the function of stress-related genes that are conserved throughout the plant kingdom. LEA (late embryogenesis abundant) proteins are highly conserved proteins that were originally discovered in desiccating seeds and were suggested to play a major role in the acquisition of seed desiccation tolerance (Battaglia et al. 2008). Stress-induced expression of *LEA* genes was also observed in vegetative tissues pointing to an additional role in stress adaptation in these tissues. Based on the primary structure of LEA proteins it is assumed that the hydrophilic disordered regions fold upon water-stress into amphipathic alpha-helices that stabilise and protect cellular membranes. Direct evidence for an essential role of LEA proteins in water stress adaptation was obtained from a *P. patens* mutant line defective in the deydrin gene *DHNA*. These mutant lines were affected in their recovery from dehydration underlining the role of LEA proteins to prevent irreversible cell damage (Saavedra et al. 2006). Functional studies in *P. patens* on another protein family (TSPO: translocator protein) that is evolutionarily conserved from bacteria to mammals and plants and acts in tetraypyrrole-transport revealed an essential role in redox homeostasis under stress conditions. A common reaction under abiotic stress conditions is the generation of detrimental reactive oxygen species (ROS) that cause peroxidation of lipids, DNA and proteins leading to irreversible cell damage. The deletion of one *P. patens TSPO* gene (*PpTSPO1*) encoding a mitochondrial membrane localised TSPO protein caused enhanced susceptibility to abiotic stress (Frank et al. 2007). The Δ*PpTSPO1* mutant lines showed elevated ROS levels that were accompanied by enhanced lipid peroxidation and cell death rates. PpTSPO1 is required for the proper allocation of tetrapyrrole intermediates between plastids and mitochondria. In the absence of PpTSPO1 highly photoreactive intermediates accumulate in the cytosol and lead to an increase in ROS. Strikingly, the *P. patens* genome encodes four additional *PpTSPO* homologues whereas *A. thaliana* encodes a single TSPO protein. It remains to be shown if TSPO proteins in flowering plants have a similar essential function in stress adaptation.

It is well known that changes in cytolsolic Ca^{2+} concentrations and the generation of specific Ca^{2+}

signatures are immediate events in stress-related signalling pathways and ATP-driven Ca^{2+}-ATPases were discussed to be major components to determine transient Ca^{2+} elevations. However, disruption of Ca^{2+}-ATPases in *A. thaliana* caused developmental abnormalities and disturbed gibberellin signalling, but had no effect on stress tolerance (Chen et al. 1997; Schiott et al. 2004; George et al. 2008). In contrast, the deletion of the Ca^{2+}-ATPase PCA1 in *P. patens* led to a reduced stress tolerance of the mutant lines pointing to an essential function of this Ca^{2+} pump in stress-induced signalling pathways (Qudeimat et al. 2008). PCA1 is localised to membranes of small vacuoles and required for the generation of a transient Ca^{2+} elevation in response to enhanced salt concentrations. The perturbed Ca^{2+} response in Δ*PCA1* mutants correlates with altered expression levels of stress-responsive genes suggesting that PCA1 is required for triggering an immediate stress response.

The functional analyses of stress-related genes in *P. patens* underline the potential of these studies in the identification and elucidation of molecular and biochemical mechanisms which contribute to the high stress tolerance in mosses. Meanwhile, the completion of the *P. patens* genome sequence facilitates the development of whole genome microarrays for gene expression profiling in response to abiotic stress factors. The use of EST-based microarrays already underlined the power of this method in the identification of a large set of stress-induced genes in *P. patens* (Cuming et al. 2007; Richardt et al. 2010). The identification of additional stress-related genes in *P. patens* and the comparison with the stress-related gene repertoire in flowering plants will provide further insight into the evolution of abiotic stress adaptation in plants. Furthermore, moss-specific mechanisms that contribute to the high degree of stress tolerance might be suitable targets for the transfer into seed plants for crop plant improvement.

High-throughput Analyses

Due to its evolutionary conserved position and the resulting potential for a discovery of genes, proteins and metabolites not present in higher plants, high-throughput analyses in *Physcomitrella patens* have become a suitable means for identifying candidate genes for targeted knock-out generation, for recombinant protein production, the so called molecular farming, or for metabolite isolation for food improvement and biofuel synthesis.

Various resources are available for a screening of candidate genes in the genome of *Physcomitrella*, the most prominent one being the cosmoss database (www.cosmoss.org) which includes the genome assembly as published in the draft genome sequence (Rensing et al. 2008), additional EST collections covering the complete life cycle (Rensing et al. 2002; Nishiyama et al. 2003; Lang et al. 2005) and informations on about 20,000 full-length cDNAs. Results, so far, indicate that the genome of *P. patens* encodes for approximately 30,000 proteins (Lang et al. 2008). Interestingly, about 10,000 of them have no reliable functional annotation, yet (unpublished). The availability of the genome sequence from *P. patens* and the publicly available database enable for a comparative analysis with the genomes from other seed plants such as *Arabidopsis thaliana*, rice and poplar, therefore contributing to the understanding of plant diversity.

Two oligonucleotide based microarrays from Combimatrix are available for *Physcomitrella*, one containing all genes coding for transcription factors and transcription-associated genes (Richardt et al. 2007) and a whole-genome array containing all the putative gene models available in the genome. A comparative set of transcription associated protein (TAP) families which are focused on, but not limited to, land plants has been determined out of the analysis of the first array in PlanTAPDB, the Plant Transcription-Associated Proteins DataBase. Besides transcription factors, extensively studied in seed plants, PlanTAPDB includes transcriptional regulators and putative TAPs and covers a broad taxonomic range including algae and the moss *P. patens*. Results from the whole-genome array, on the contrary, are envisaged to be included in cosmoss in an expression atlas, which will allow for an expression profile analysis of genes under different experimental conditions, i.e. in response to abiotic stress or phytohormones.

Besides the analysis of single genes via the generation of knockout mutants the application of forward-genetic approaches is essential for the investigation of multiple gene effects. For this purpose a mutant collection was created which allows for a subsequent characterisation of aberrant phenotypes and an identification of the mutated genomic locus. Ideally, mutagenesis should not be directed and all the genes of an organism should have the same probability of being targeted, thus leading to the generation of a saturated mutant collection. The isolation of targeted genes is easier if mutagenesis is done via the integration of known DNA sequences. Such an approach was established for *Physcomitrella*, for which cloned cDNA regions were mutated via transposon mutagenesis and then implemented in a high-throughput approach generating 160 transgenic lines per day. From 16,000 analysed *Physcomitrella* mutants about 16% displayed a phenotype deviating from wild-type (Egener et al. 2002). This value is way over the rate of comparable mutant collections of the flowering model plant *A. thaliana*. The large number of *Physcomitrella* mutants with an aberrant

gametophytic phenotype is most likely the result of the haploid status of the gametophyte. Besides from mutants with a disrupted development many auxotropic mutants showing metabolic defects could be isolated using a high throughput metabolic screen (Schulte et al. 2006). This large-scale analysis performed for 51,180 targeted knockout and the characterisation of the mutants might help to find genes with essential functions in plant specific biosynthetic pathways, like vitamin synthesis or the unsaturated fatty acid metabolism. These genes therefore are candidates for future biotechnological applications.

The sequenced genome of *Physcomitrella* was instrumental for a quick and reliable identification of proteins due to an automated sequence comparison of mass spectrometry-identified peptides via the cosmoss database. Large amounts of moss are necessary for such shot-gun proteomics experiments but as cultivation of *P. patens* under standardised conditions in the bioreactor or in bubble flasks can easily be up-scaled this point does not represent a limiting step. Methods for the selective isolation of organelles from *P. patens* with a subsequent extraction of proteins from these compartments, as well as well-established methods for the investigation of signal transduction pathways are available. However, there are still some requirements needed for a high-throughput analysis based on proteomics data. For shot-gun proteomics approaches as used e.g. for the mapping of organellar proteomes from plastids and mitochondria there is a need for automated bioinformatics tools for data evaluation and data analysis. Furthermore, the mass spectrometric detection is dependent on the detection technique thus generating results of varying quality, something which has to be taken into account in the subsequent data mining processes and while setting up the final list of reliably identified proteins. The characterisation of signal transduction processes and the analysis of changes in the phosphorylation of members within a pathway are less prone to bioinformatics difficulties but encounters experimental limitations which require the combination of diverse methods and the adaption of protocols depending on the topic to be addressed. Enrichment and identification of phosphorylated proteins in *P. patens* therefore requires fast handling of samples, since phosphorylations usually are unstable modifications, and a combination of reverse-phase chromatography, metal affinity chromatography, capillary electrophoresis, LC-MS/MS and MALDI-TOF-MS. By this means about 250 phosphopeptides have been identified in *Physcomitrella* (Heintz et al. 2004).

Moss Bioinformatics and Genomics

The publication record of bioinformatics and genomics in Bryopsida is scarce and has been primarily focussed on the Funariaceae molecular model *Physcomitrella patens*. EST projects were pursued for the desiccation tolerant Pottiaceae *Syntrichia ruralis* (Oliver et al. 2004) [9,991 ESTs], cosmopolitan Ditrichaceae *Ceratodon purpureus* [1,677 ESTs] and the arctic Aulacomniaceae *Aulacomnium turgidum* [439 ESTs]. Only the *Syntrichia* data were analyzed on a larger scale via assembly, expression profiling and functional annotation (Oliver et al. 2004). But with the advent and cost-effectiveness of (ultra-) high-throughput sequencing technologies like e.g. 454, Illumina and SOLID, a broader coverage of the ecological complexity of mosses and other bryophytes is within our grasp. The analysis of the plastid genome of *S. ruralis* (Genbank Refseq NC_012052) and several EST projects of Bryopsida are underway. The upcoming and future bryophyte genomics and transcriptomics projects will greatly benefit from the well established resources that have been created for the model moss *P. patens* which we will review in the following.

Initial sequencing studies of *P. patens* genes date back to the late nineties. Genes with significant sequence similarity to annotated genes of seed plants, as well as putative species-specific genes were found by sequencing cDNAs isolated by subtractive hybridization (Reski et al. 1998). Later, sequencing of cDNAs from tissue treated with abscisic acid (ABA) revealed the same and also demonstrated that the same classes of stress-induced genes were activated in moss as those known from flowering plants (Machuka et al. 1999). Subsequently, several large-scale EST sequencing projects were undertaken around the globe (e.g. UK, The Physcomitrella EST programme; USA; Germany (Rensing et al. 2002), http://www.cos-moss.org and Japan (Nishiyama et al. 2003), http://moss.nibb.ac.jp/). Some of the libraries were normalized and/or subtracted and together with all available data assembled and annotated into a virtual transcriptome representation of low redundancy (Lang et al. 2005).

To allow functional characterization of this vast amount of data, a high quality functional annotation pipeline has been developed and applied to annotate approximately 63% of the virtual transcripts (Lang et al. 2005). In addition, a web interface has been developed that allows easy access to the virtual transcriptome of *Physcomitrella* and the partial transcriptomes of *S. ruralis* and *C. purpureus* (www.cosmoss.org). To date, nearly 400,000 ESTs have been generated from libraries spanning the complete lifecycle of *P. patens* and are available as

the virtual transcriptome representation 04/09 (www.cosmoss.org).

Both organellar genomes, the mitochondrial (Terasawa et al. 2007) and the chloroplast (Sugiura et al. 2003) genome, are fully sequenced and have already revealed valuable insights into the evolution of Plantae (Qiu et al. 2006; Turmel et al. 2006; Turmel et al. 2007).

In order to further establish *P. patens* as molecular model for functional comparative genomics of embryophytes, Rensing et al. (2002) suggested that "*this is a good time to establish an international moss-genome-sequencing project*". At the annual moss meeting in 2004 in Freiburg the International Moss Genome Consortium was initiated and plans to sequence the genome were formed and pursued since then.

The genome has been sequenced as part of the U.S. Department of Energy's community sequencing program by a whole genome shotgun approach at the Joint Genome Institute (JGI) in 2005. The haploid genome of *P. patens* has an estimated size of ~510Mbp (C-value: 0.53pg) and is organized in 27 small chromosomes (Reski et al. 1994; Schween et al. 2003). The draft sequence was published in early 2008 (Rensing et al. 2008) and as expected, revealed valuable insights into the conquest of land by plants. From v1.1 of the *P. patens* genome ~36,000 protein-encoding genes were predicted (Rensing et al. 2008), whereas clustered EST (expressed sequence tags) data suggested only ~25,000 protein-encoding moss genes (Rensing et al. 2002; Lang et al. 2005; Rensing et al. 2005). More detailed analysis prompted the exclusion of gene models overlapping with transposable elements, tRNA- and microRNA-genes prior the release of v1.2 of the *P. patens* genome. From this, we can predict 27,949 protein-encoding *P. patens* genes (Lang et al. 2008).

This provides us with an additional example of the often quoted "C-value paradox" that the genome of an inconspicuous moss harbours a complexity in terms of size, repeat content and in number of protein-encoding genes (~30,000) which is in the same range as those of morphologically more complex organisms, such as *Homo sapiens* or *A. thaliana* (Sterck et al. 2007).

With the current draft we have an initial overview of the genomic structure and a good understanding of the complement of protein-coding and non-protein-coding regions of a haploid moss genome. The internet resource http://www.cosmoss.org provides access to the past, current (v1.2) and future versions of the *P. patens* genome and annotation using BLAST, sequence retrieval and an integrated genome browser including manual annotation interfaces. The international efforts to finish the *P. patens* in form of a reference genome (v2.0), i.e. assembling into the 27 pseudo-molecules representing the 27 chromosomes, are ongoing.

Production of complex Biopharmaceuticals with Moss Bioreactors

Recombinant proteins become increasingly important within the pharmaceutical industry. Because of their complex nature, proteins hardly can be produced by chemical synthesis but have to be synthesized biotechnologically in living cells or organisms. For this reason, recombinantly produced pharmaceutical proteins caught the name "biopharmaceuticals" or "biologics", respectively. Nowadays, the vast majority of biopharmaceuticals have been produced in microbial or mammalian cells. Microbial systems are clearly superior in terms of easy cultivation and high product yields, whereas mammalian cell lines (preferentially Chinese Hamster Ovary cells) are favoured for complex multimeric proteins or those requiring posttranslational modifications (Schmidt 2004; Walsh & Jefferis 2006). In comparison to the currently used systems, plants combine several advantages making them interesting alternatives. As higher eukaryotes, they perform posttranslational modifications closely resembling those of humans and the risk of product contamination by infectious agents deriving from the used cells or media is neglectable (Fischer et al. 2004; Ma et al. 2005). However, only plant-based systems cultivated in vitro allow the precisely controlled environment which is required to produce pharmaceuticals under so-called GMP (Good Manufacturing Practices) conditions (Hellwig et al. 2004). While feasibility studies for plants as expression platforms for recombinant biopharmaceuticals were successfully performed almost twenty years ago (Düring et al. 1990; Sijmons et al. 1990) and several plant-derived biopharmaceuticals have been submitted for clinical trials in humans most plant systems are still limited in producing correctly glycosylated proteins. Protein Asparagine (N)-linked glycosylation is a frequent modification of the majority of proteins from human blood (e.g. antibodies, growth factors, hormones) which displays the main candidates as biopharmaceuticals. Plants attach sugar chains to proteins which resemble mammalian glycosylation most of all alternative production systems, however, plant glycans contain sugar moieties which were shown to activate the human immune system, the most critical issue when a pharmaceutical is applied to patients (Garcia-Casado et al. 1996; van Ree et al. 2000; Bardor et al. 2003; Faye et al. 2005).

Some years ago, the *P. patens* was suggested and commercialized as an alternative production platform that meets this concern by providing an exceptional

accessibility for precise genetic modifications via highly efficient mitotic homologous recombination (Strepp et al. 1998; Kamisugi et al. 2006; Decker & Reski 2008). In-vitro cultivation of protonema and gametophores has been established since decades (Reski & Abel 1985) and efficient transformation protocols are also available for many years (Rother et al. 1994; Frank et al. 2005). Highly controllable and standardized cultivation conditions for biopharmaceutical production were reached in different photobioreactors developed as stirred glass tanks for a volume up to 15 L (Hohe & Reski 2002, Fig. 2) or in a modular, fully scalable glass tubular reactor (Lucumi et al. 2005; Lucumi & Posten 2006; Perner-Nochta et al. 2007). A 30-L tubular pilot reactor was employed to determine optimal parameters for batch (Lucumi et al. 2005) as well as continuous cultivation (Lucumi & Posten 2006). Recent improvements of the tubular system focused on the establishment of a modular 100-L bioreactor unit allowing the manufacture of biopharmaceuticals for clinical studies (www.greenovation.com).

Genetic tools for enhancing recombinant protein production focused on strong and tunable gene expression systems using *P. patens*-derived as well as heterologous promoter cassettes (Horstmann et al. 2004; Jost et al. 2005; Gitzinger et al. 2009). In addition, the efficiency of product secretion was improved by a screening of various secretions signals in which *P. patens* signal sequences turned out to be superior to heterologous (i.e. human) ones (Schaaf et al. 2005; Weise et al. 2006). Secretion of *P. patens*-produced biopharmaceuticals to the culture supernatant is favoured for two main reasons: It allows continuous harvest of products from a simple mineral medium which contains only a very few moss proteins and no additives. Thus, the isolation and purification (the so-called downstream processing) of the recombinant products is eased compared to other systems. In addition, a secretory system is desirable in order to navigate the nascent proteins through the endoplasmic reticulum and the Golgi apparatus which are responsible for N-glycosylation and many additional posttranslational modifications. These compartments are passed by the vast majority of extracellular proteins.

As mentioned above, correct protein N-glycosylation is a frequent and very important protein modification on biopharmaceuticals and differences in single sugar residues may result in functional aberrations or adverse reactions of the human body against the pharmaceutical protein. Glyco-engineering of *P. patens*-produced biopharmaceuticals was achieved via targeted knockouts of genes coding for those glycosyltransferases which attach the two most critical sugar molecules to the growing glycan chain (Koprivova et al. 2004). In addition, the mechanism for unwished terminal beta-1,3 galactosylation was identified (Launhardt et al. 2008) and a human beta-1,4 galactosyltransferase gene, unknown from plants at all, was introduced into the moss genome via homologous recombination (Huether et al. 2005). The glyco-engineered *P. patens* strains were shown to be completely devoid of allergenic sugar residues and were employed to synthesise several products of pharmaceutical value, including human VEGF (Koprivova et al. 2004), IgG class antibodies (Gorr & Jost 2005; Nechansky et al. 2007; Schuster et al. 2007), and erythropoietin (Weise et al. 2007). For some antibodies, probably the most interesting and largest group of biopharmaceuticals in use and clinical development, glyco-engineered *P. patens* strains turned out to be superior to traditional mammalian production hosts. The pharmacological efficiency of some antibodies is rather disappointing due to weak antibody-dependent cellular cytotoxicity (ADCC), an important effector function of antibodies. ADCC is mediated by receptor binding of IgG-class antibodies and was enhanced with recombinant antibodies produced in glyco-engineered moss up to 40-fold compared to the parental antibody produced in mammalian cells (Nechansky et al. 2007; Schuster et al. 2007), impressively demonstrating the suitability of *P. patens* to produce biopharmaceuticals with enhanced product quality.

International Moss Stock Center

More and more bryophyte species are collected from the wild and transferred to axenic culture, which is the prerequisite of any molecular analysis (Hohe & Reski 2005). In addition, the pure number of genetically

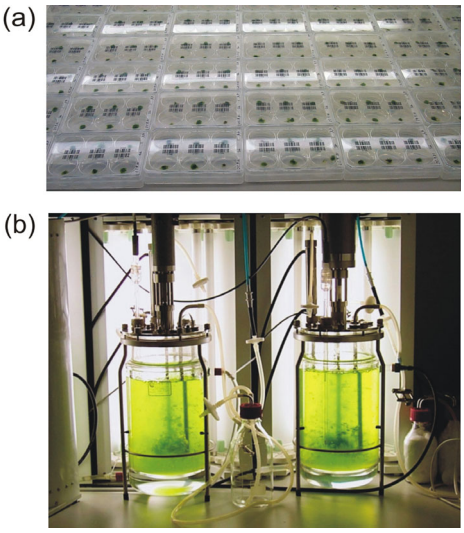

Figure 2. In-vitro cultivation of *Physcomitrella patens* for biotechnology. (a) Large-scale mutant collection of transformed *P. patens* strains, grown on solid medium. (b) Propagation of protonema in stirred glass-tank photobioreactors.

engineered moss mutants is rapidly growing. To enhance the reproducibility between the research in different laboratories, to promote the exchange of scientific material, and last but not least, to meet the high standards of high profile journals, a central resource is needed, that stores and preserves any bryophyte strain, wild type or mutant, that has been used for molecular analyses and has been published in peer-reviewed journals. In contrast to most seed plants, bryophytes can be preserved deep-frozen in or over liquid nitrogen. This method was optimized by Schulte & Reski (2004) for a huge amount of *Physcomitrella* mutants, and is also applicable to a variety of other axenic cultures of different bryophytes (unpublished). Even after ten years deep-frozen, moss mutants can be thawed and re-grown unchanged since then to 100% (unpublished). Recently, this technique was used to establish the International Moss Stock Center (IMSC), based at the University of Freiburg, but operating world-wide. The IMSC can be accessed via www.moss-stock-center.org.

Conclusions

The potential of bryophytes for applied research with implications for agriculture and for human health still is not fully appreciated and explored. Nevertheless, the work of Jan-Peter Frahm and others have shown that bryotechnology is a fast developing sector of biotechnology in general.

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References

Ando, H. & A. Matsuo 1984. Applied bryology, pp. 133-224. In W. Schultze-Motel (ed.), *Advances in Bryology 2* Cramer, Vaduz.

Anterola, A., E. Shanle, K. Mansouri, S. Schuette & K. Renzaglia 2009. Gibberellin precursor is involved in spore germination in the moss *Physcomitrella patens*. *Planta* 229: 1003-1007.

Asakawa, Y. 1981. Biologically active substances obtained from bryophytes. *Journal of the Hattori Botanical Laboratory* 50: 123-142.

Asakawa, Y. 2007. Biologically active compounds from bryophytes*. *Pure and Applied Chemistry* 79: 557–580.

Bardor, M., C. Faveeuw, A. C. Fitchette, D. Gilbert, L. Galas, F. Trottein, L. Faye & P. Lerouge 2003. Immunoreactivity in mammals of two typical plant glyco-epitopes, core alpha(1,3)-fucose and core xylose. *Glycobiology* 13: 427-434.

Basile, A., S. Giordano, J. A. López-Sáez & R. Castaldo Cobianchi 1999. Antibacterial activity of pure flavonids isolated from mosses. *Phytochemistry* 52: 1479-1482.

Battaglia, M., Y. Olvera-Carrillo, A. Garciarrubio, F. Campos & A. A. Covarrubias 2008. The enigmatic LEA proteins and other hydrophilins. *Plant Physiology* 148: 6-24.

Benito, B. & A. Rodriguez-Navarro 2003. Molecular cloning and characterization of a sodium-pump ATPase of the moss *Physcomitrella patens*. *The Plant Journal* 36: 382-389.

Chen, X., M. Chang, B. Wang & B. Wu 1997. Cloning of a Ca(2+)-ATPase gene and the role of cytosolic Ca2+ in the gibberellin-dependent signaling pathway in aleurone cells. *The Plant Journal* 11: 363-371.

Cuming, A. C., S. H. Cho, Y. Kamisugi, H. Graham & R. S. Quatrano 2007. Microarray analysis of transcriptional responses to abscisic acid and osmotic, salt, and drought stress in the moss, *Physcomitrella patens*. *New Phytologist* 176: 275-287.

Dayan, F. E. & J. G. Romagni 2001. Lichen as a potential source of pesticides. *Pesticide Outlook* 12: 229 – 232.

Decker, E. L. & R. Reski 2008. Current achievements in the production of complex biopharmaceuticals with moss bioreactors. *Bioprocess Biosystems Engineering* 31: 3-9.

Dikshit, A. D., D. K. Pandey & S. Nath 1982. Antifungal activity of some bryophytes against human pathogens. *Journal of Indian Botanical Society* 61: 447-448.

Ding, H. 1982. Medical spore-bearing plants of China. 499 pp. Shanghai.

Düring, K., S. Hippe, F. Kreuzaler & J. Schell 1990. Synthesis and self-assembly of a functional monoclonal antibody in transgenic *Nicotiana tabacum*. *Plant Molecular Biology* 15: 281-293.

Egener, T., J. Granado, M. C. Guitton, A. Hohe, H. Holtorf, J. M. Lucht, S. A. Rensing, K. Schlink, J. Schulte, G. Schween, S. Zimmermann, E. Duwenig, B. Rak & R. Reski 2002. High frequency of phenotypic deviations in *Physcomitrella patens* plants transformed with a gene-disruption library. *BMC Plant Biology* 2: 6.

Faye, L., A. Boulaflous, M. Benchabane, V. Gomord & D. Michaud 2005. Protein modifications in the plant secretory pathway: current status and practical implications in molecular pharming. *Vaccine* 23: 1770-1778.

Fischer, R., E. Stoger, S. Schillberg, P. Christou & R. M. Twyman 2004. Plant-based production of biopharmaceuticals. *Current Opinion in Plant Biology* 7: 152-158.

Frahm, J.-P. 2001. Biologie der Moose. Spektrum Akademischer Verlag, Heidelberg und Berlin.

Frahm, J.-P. & K. Kirchhoff 2002. Antifeedant effects of bryophyte extracts from *Neckera crispa* and *Porella obtusata* against the slug *Aarion lusitanicus*. *Cryptogamie Bryologie* 23: 271-275.

Frahm, J.-P. 2004. Recent developments of commercial products from bryophytes. *The Bryologist* 107: 277-283.

Fraile-Escanciano, A., B. Garciadeblas, A. Rodriguez-Navarro & B. Benito 2009. Role of ENA ATPase in Na(+) efflux at high pH in bryophytes. *Plant Molecular Biology* 71: 599-608

Frank, W., K. M. Baar, E. Qudeimat, M. Woriedh, A. Alawady, D. Ratnadewi, L. Gremillon, B. Grimm & R. Reski 2007. A mitochondrial protein homologous to the mammalian peripheral-type benzodiazepine receptor is essential for stress adaptation in plants. *The Plant Journal* 51: 1004-1018.

Frank, W., E. L. Decker & R. Reski 2005. Molecular tools to study *Physcomitrella patens*. *Plant Biology*. 7: 220-227.

Frank, W., D. Ratnadewi & R. Reski 2005. *Physcomitrella patens* is highly tolerant against drought, salt and osmotic stress. *Planta* 220: 384-394.

Garcia-Casado, G., R. Sanchez-Monge, M. J. Chrispeels, A. Armentia, G. Salcedo & L. Gomez 1996. Role of complex asparagine-linked glycans in the allergenicity of plant glycoproteins. *Glycobiology* 6: 471-477.

George, L., S. M. Romanowsky, J. F. Harper & R. A. Sharrock 2008. The ACA10 Ca2+-ATPase regulates adult vegetative development and inflorescence architecture in Arabidopsis. *Plant Physiology* 146: 716-728.

Gitzinger, M., J. Parsons, R. Reski & M. Fussenegger 2009. Functional cross-kingdom conservation of mammalian and moss (*Physcomitrella patens*) transcription, translation and secretion machineries. *Plant Biotechnology* J 7: 73-86.

Glime, J. M. 2007. Bryophyte Ecology. Volume 1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. http://www.bryoecol.mtu.edu/

Gorr, G. & W. Jost 2005. Glycosylation design in transgenic moss for better product efficacy. *Bioprocessing Journal* 4: 26-30.

Gulabani, A. 1974. Bryophytes as economic plants. *Botanica* 14: 73-75.

Hedwig, D. J. 1793. D. Johann Hedwig's Sammlung seiner zerstreuten Abhandlungen und Beobachtungen über botanisch ökonomische Gegenstände. Erstes Bändchen mit fünf illuminierten Kupfertafeln. 208 p. Siegfried Lebrecht Crusius, Leipzig.

Heintz, D., V. Wurtz, A. A. High, A. Van Dorsselaer, R. Reski & E. Sarnighausen 2004. An efficient protocol for the identification of protein phosphorylation in a seedless plant, sensitive enough to detect members of signalling cascades. *Electrophoresis* 25: 1149-1159.

Hellwig, S., J. Drossard, R. M. Twyman & R. Fischer 2004. Plant cell cultures for the production of recombinant proteins. *Nature Biotechnology* 22: 1415-1422.

Hohe, A. and R. Reski 2002. Optimisation of a bioreactor culture of the moss *Physcomitrella patens* for mass production of protoplasts. *Plant Science* 163: 69-74.

Hohe, A. & R. Reski 2005. From axenic spore germination to molecular farming: one century of bryophyte in vitro culture. *Plant Cell Reports* 23: 513-521.

Horstmann, V., C. M. Huether, W. Jost, R. Reski & E. L. Decker 2004. Quantitative promoter analysis in *Physcomitrella patens*: a set of plant vectors activating gene expression within three orders of magnitude. *BMC Biotechnology* 4: 13.

Huether, C. M., O. Lienhart, A. Baur, C. Stemmer, G. Gorr, R. Reski & E. L. Decker 2005. Glyco-engineering of moss lacking plant-specific sugar residues. *Plant Biology* 7: 292-299.

Jost, W., S. Link, V. Horstmann, E. L. Decker, R. Reski & G. Gorr 2005. Isolation and characterisation of three moss-derived beta-tubulin promoters suitable for recombinant expression. *Current Genetics* 47: 111-120.

Kamisugi, Y., K. Schlink, S. A. Rensing, G. Schween, M. von Stackelberg, A. C. Cuming, R. Reski & D. J. Cove 2006. The mechanism of gene targeting in *Physcomitrella patens*: homologous recombination, concatenation and multiple integration. *Nucleic Acids Research* 34: 6205-6214.

Koprivova, A., C. Stemmer, F. Altmann, A. Hoffmann, S. Kopriva, G. Gorr, R. Reski & E. L. Decker 2004. Targeted knockouts of *Physcomitrella* lacking plant-specific immunogenic N-glycans. *Plant Biotechnology Journal* 2: 517-523.

Lang, D., J. Eisinger, R. Reski & S. A. Rensing 2005. Representation and high-quality annotation of the *Physcomitrella patens* transcriptome demonstrates a high proportion of proteins involved in metabolism among mosses. *Plant Biology* 7: 228-237.

Lang, D., A. D. Zimmer, S. A. Rensing & R. Reski 2008. Exploring plant biodiversity: the *Physcomitrella* genome and beyond. *Trends in Plant Science* 13: 542-549.

Launhardt, H., C. Stemmer, W. Jost, G. Gorr, R. Reski & S. Rensing 2008. Galactosyltransferase. Patent WO2008037490.

Lucumi, A. & C. Posten 2006. Establishment of long-term perfusion cultures of recombinant moss in a pilot tubular photobioreactor. *Process Biochemistry* 41: 2180-2187.

Lucumi, A., C. Posten & M. N. Pons 2005. Image analysis supported moss cell disruption in photo-bioreactors. *Plant Biology* 7: 276-282.

Ma, J. K., E. Barros, R. Bock, P. Christou, P. J. Dale, P. J. Dix, R. Fischer, J. Irwin, R. Mahoney, M. Pezzotti, S. Schillberg, P. Sparrow, E. Stoger & R. M. Twyman 2005. Molecular farming for new drugs and vaccines. Current perspectives on the production of pharmaceuticals in transgenic plants. *EMBO Reports* 6: 593-599.

Machuka, J., S. Bashiardes, E. Ruben, K. Spooner, A. Cuming, C. Knight & D. Cove 1999. Sequence analysis of expressed sequence tags from an ABA-treated cDNA library identifies stress response genes in the moss *Physcomitrella patens*. *Plant and Cell Physiology* 40: 378-387.

Merkuria, T., U. Steiner, H. Hindorf, J.-P. Frahm & H.-W. Dehne 2005. Bioactivity of bryophyte extracts against *Botrytis cinerea*, *Alternaria solani* and *Phytophtora infestans*. *Journal of Applied Botany and Food Quality* 79: 89-93.

Nechansky, A., M. Schuster, W. Jost, P. Siegl, S. Wiederkum, G. Gorr & R. Kircheis 2007. Compensation of endogenous IgG mediated inhibition of antibody-dependent cellular cytotoxicity by glyco-engineering of therapeutic antibodies. *Molecular Immunology* 44: 1826-1828.

Nishiyama, T., T. Fujita, I. T. Shin, M. Seki, H. Nishide, I. Uchiyama, A. Kamiya, P. Carninci, Y. Hayashizaki, K. Shinozaki, Y. Kohara & M. Hasebe 2003. Comparative genomics of *Physcomitrella patens* gametophytic transcriptome and *Arabidopsis thaliana*: implication for land plant

evolution. *The Proceedings of the National Academy of Science U S A* 100: 8007-8012.

Oliver, M. J., S. E. Dowd, J. Zaragoza, S. A. Mauget & P. R. Payton 2004. The rehydration transcriptome of the desiccation-tolerant bryophyte Tortula ruralis: transcript classification and analysis. *BMC Genomics* 5: 89.

Oliver, M. J., J. Hudgeons, S. E. Dowd & P. R. Payton 2009. A combined subtractive suppression hybridization and expression profiling strategy to identify novel desiccation response transcripts from *Tortula ruralis* gametophytes. *Physiologia plantarum* 136: 437-460.

Pavletic, Z. & B. Stilinovic 1963. Untersuchungen über die antibiotische Wirkung von Moosextrakten auf einige Bakterien. *Acta Botanica Croatica* 22: 133-139.

Perner-Nochta, I., A. Lucumi & C. Posten 2007. Photoautotrophic cell and tissue culture in a tubular photobioreactor. *Engineering in Life Sciences* 7: 127-135.

Qiu, Y. L., L. Li, B. Wang, Z. Chen, V. Knoop, M. Groth-Malonek, O. Dombrovska, J. Lee, L. Kent, J. Rest, G. F. Estabrook, T. A. Hendry, D. W. Taylor, C. M. Testa, M. Ambros, B. Crandall-Stotler, R. J. Duff, M. Stech, W. Frey, D. Quandt & C. C. Davis 2006. The deepest divergences in land plants inferred from phylogenomic evidence. *The Proceedings of the National Academy of Science U S A* 103: 15511-15516.

Qudeimat, E., A. M. Faltusz, G. Wheeler, D. Lang, C. Brownlee, R. Reski & W. Frank 2008. A PIIB-type Ca2+-ATPase is essential for stress adaptation in *Physcomitrella patens*. *The Proceedings of the National Academy of Science U S A* 105: 19555-19560.

Rensing, S. A., D. Fritzowsky, D. Lang & R. Reski 2005. Protein encoding genes in an ancient plant: analysis of codon usage, retained genes and splice sites in a moss, *Physcomitrella patens*. *BMC Genomics* 6: 43.

Rensing, S. A., D. Lang, A. D. Zimmer, A. Terry, A. Salamov, H. Shapiro, T. Nishiyama, P. F. Perroud, E. A. Lindquist, Y. Kamisugi, T. Tanahashi, K. Sakakibara, T. Fujita, K. Oishi, I. T. Shin, Y. Kuroki, A. Toyoda, Y. Suzuki, S. Hashimoto, K. Yamaguchi, S. Sugano, Y. Kohara, A. Fujiyama, A. Anterola, S. Aoki, N. Ashton, W. B. Barbazuk, E. Barker, J. L. Bennetzen, R. Blankenship, S. H. Cho, S. K. Dutcher, M. Estelle, J. A. Fawcett, H. Gundlach, K. Hanada, A. Heyl, K. A. Hicks, J. Hughes, M. Lohr, K. Mayer, A. Melkozernov, T. Murata, D. R. Nelson, B. Pils, M. Prigge, B. Reiss, T. Renner, S. Rombauts, P. J. Rushton, A. Sanderfoot, G. Schween, S. H. Shiu, K. Stueber, F. L. Theodoulou, H. Tu, Y. Van de Peer, P. J. Verrier, E. Waters, A. Wood, L. Yang, D. Cove, A. C. Cuming, M. Hasebe, S. Lucas, B. D. Mishler, R. Reski, I. V. Grigoriev, R. S. Quatrano & J. L. Boore 2008. The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. *Science* 319: 64-69.

Rensing, S. A., S. Rombauts, Y. Van de Peer & R. Reski 2002. Moss transcriptome and beyond. *Trends in Plant Science* 7: 535-538.

Reski, R. 1998. Development, genetics and molecular biology of mosses. *Botanica Acta* 111: 1-15.

Reski, R. & W. O. Abel 1985. Induction of budding on chloronemata and caulonemata of the moss, *Physcomitrella patens*, using isopentenyladenine. *Planta* 165: 354-358.

Reski, R., M. Faust, X. H. Wang, M. Wehe and W. O. Abel 1994. Genome analysis of the moss *Physcomitrella patens* (Hedw.) B.S.G. *Molecular and General Genetics* 244: 352-359.

Reski, R., S. Reynolds, M. Wehe, T. Kleber-Janke and S. Kruse 1998. Moss (*Physcomitrella patens*) expressed sequence tags include several sequences which are novel for plants. *Botanica Acta* 111: 143-149.

Richardt, S., D. Lang, W. Frank, R. Reski and S. A. Rensing 2007. PlanTAPDB: A phylogeny-based resource of plant transcription associated proteins. *Plant Physiology* 143: 1452-1466.

Richardt, S., G. Timmerhaus, D. Lang, E. Qudeimat, L. G. G. Corrêa, R. Reski, S. A. Rensing and W. Frank 2010. Microarray analysis of the moss *Physcomitrella patens* reveals evolutionarily conserved transcriptional regulation of salt stress and abscisic acid signalling. *Plant Molecular Biology* 72: 27–45.

Rother, S., B. Hadeler, J. M. Orsini, W. O. Abel and R. Reski 1994. Fate of a mutant macrochloroplast in somatic hybrids. *Journal of Plant Physiology* 143: 72-77.

Saavedra, L., J. Svensson, V. Carballo, D. Izmendi, B. Welin and S. Vidal 2006. A dehydrin gene in *Physcomitrella patens* is required for salt and osmotic stress tolerance. *The Plant Journal* 45: 237-249.

Schaaf, A., S. Tintelnot, A. Baur, R. Reski, G. Gorr and E. L. Decker 2005. Use of endogenous signal sequences for transient production and efficient secretion by moss (*Physcomitrella patens*) cells. *BMC Biotechnology* 5: 30.

Schiott, M., S. M. Romanowsky, L. Baekgaard, M. K. Jakobsen, M. G. Palmgren & J. F. Harper 2004. A plant plasma membrane Ca2+ pump is required for normal pollen tube growth and fertilization. *The Proceedings of the National Academy of Science U S A* 101: 9502-9507.

Schmidt, F. R. 2004. Recombinant expression systems in the pharmaceutical industry. *Applied Microbiology and Biotechnology* 65: 363-372.

Schulte, J. & R. Reski 2004. High-throughput cryopreservation of 140,000 *Physcomitrella patens* mutants. *Plant Biology* 6: 119-127.

Schulte, J., A. Erxleben, G. Schween & R. Reski 2006. High throughput metabolic screen of *Physcomitrella* transformants. *The Bryologist* 109: 247-256.

Schuster, M., W. Jost, G. C. Mudde, S. Wiederkum, C. Schwager, E. Janzek, F. Altmann, J. Stadlmann, C. Stemmer & G. Gorr 2007. In vivo glyco-engineered antibody with improved lytic potential produced by an innovative non-mammalian expression system. *Biotechnology Journal* 2: 700-708.

Schween, G., G. Gorr, A. Hohe & R. Reski 2003. Unique tissue-specific cell cycle in *Physcomitrella*. *Plant Biology* 5: 50-58.

Sijmons, P. C., B. M. Dekker, B. Schrammeijer, T. C. Verwoerd, P. J. van den Elzen & A. Hoekema 1990. Production of correctly processed human

serum albumin in transgenic plants. *Biotechnology (N Y)* 8: 217-221.

Sterck, L., S. Rombauts, K. Vandepoele, P. Rouze & Y. Van de Peer 2007. How many genes are there in plants (… and why are they there)? *Current Opinion in Plant Biology* 10: 199-203.

Strepp, R., S. Scholz, S. Kruse, V. Speth and R. Reski 1998. Plant nuclear gene knockout reveals a role in plastid division for the homolog of the bacterial cell division protein FtsZ, an ancestral tubulin. *Proceedings of the National Academy of Science U S A* 95: 4368-4373.

Sugiura, C., Y. Kobayashi, S. Aoki, C. Sugita & M. Sugita 2003. Complete chloroplast DNA sequence of the moss *Physcomitrella patens*: evidence for the loss and relocation of rpoA from the chloroplast to the nucleus. *Nucleic Acids Research* 31: 5324-5331.

Terasawa, K., M. Odahara, Y. Kabeya, T. Kikugawa, Y. Sekine, M. Fujiwara & N. Sato 2007. The mitochondrial genome of the moss *Physcomitrella patens* sheds new light on mitochondrial evolution in land plants. *Molecular Biology and Evolution* 24: 699-709.

Turmel, M., C. Otis & C. Lemieux 2006. The chloroplast genome sequence of *Chara vulgaris* sheds new light into the closest green algal relatives of land plants. *Molecular Biology and Evolution* 23: 1324-1338.

Turmel, M., C. Otis & C. Lemieux 2007. An unexpectedly large and loosely packed mitochondrial genome in the charophycean green alga *Chlorokybus atmophyticus*. *BMC Genomics* 8: 137.

van Ree, R., M. Cabanes-Macheteau, J. Akkerdaas, J. P. Milazzo, C. Loutelier-Bourhis, C. Rayon, M. Villalba, S. Koppelman, R. Aalberse, R. Rodriguez, L. Faye & P. Lerouge 2000. Beta(1,2)-xylose and alpha(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. *The Journal of Biological Chemistry* 275: 11451-11458.

Von Schwartzenberg, K., W. Schultze & H. Kassner 2004. The moss *Physcomitrella patens* releases a tetracyclic diterpene. *Plant Cell Reports* 22: 780-786.

Walsh, G. and R. Jefferis 2006. Post-translational modifications in the context of therapeutic proteins. *Nature Biotechnology* 24: 1241-1252.

Weise, A., F. Altmann, M. Rodriguez-Franco, E. R. Sjoberg, W. Baumer, H. Launhardt, M. Kietzmann & G. Gorr 2007. High-level expression of secreted complex glycosylated recombinant human erythropoietin in the Physcomitrella Delta-fuc-t Delta-xyl-t mutant. *Plant Biotechnology Journal* 5: 389-401.

Weise, A., M. Rodriguez-Franco, B. Timm, M. Hermann, S. Link, W. Jost & G. Gorr 2006. Use of *Physcomitrella patens* actin 5' regions for high transgene expression: importance of 5' introns. *Applied Microbiology and Biotechnology* 70: 337-345.

Wolters, B. 1964. Die Verbreitung antifungaler Eigenschaften bei Moosen. *Planta* 62: 88-96.

Wood, A. J., R. J. Duff & M. J. Oliver 1999. Expressed sequence tags (ESTs) from desiccated Tortula ruralis identify a large number of novel plant genes. *Plant and Cell Physiology* 40: 361-368.

Wood, A. J., R. J. Duff & M. J. Oliver 2000. The translational apparatus of Tortula ruralis: polysomal retention of transcripts encoding the ribosomal proteins RPS14, RPS16 and RPL23 in desiccated and rehydrated gametophytes. *Journal of Experimental Botany* 51: 1655-1662.

Zhu, R.-L., D. Wang, L. Xu, R.-P. Shi, J. Wang & M. Zheng 2006. Antibacterial activity in extracts of some bryophytes from China and Mongolia. *The Journal of the Hattori Botanical Laboratory* 100: 603-615.

Zinsmeister, H. D., H. Becker & T. Eicher 1991. Moose, eine Quelle biologisch aktiver Naturstoffe? *Angewandte Chemie* 103: 134-151.