Mosses in biotechnology
Eva L Decker¹ and Ralf Reski¹,²,³

Biotechnological exploitation of mosses has several aspects, for example, the use of moss extracts or the whole plant for diverse industrial applications as well as their employment as production platforms for valuable metabolites or pharmaceutical proteins, especially using the genetically and developmentally best-characterised model moss Physcomitrella patens. Whole moss plants, in particular peat mosses (Sphagnum spec.), are useful for environmental approaches, biomonitoring of environmental pollution and CO₂-neutral ‘farming’ on rewetted bogs to combat climate change. In addition, the lifestyle of mosses suggests the evolution of genes necessary to cope withiotic and abiotic stress situations, which could be applied to crop plants, and their structural features bear an inspiring potential for biomimetics approaches.

Addresses
¹ Plant Biotechnology, Faculty of Biology, University of Freiburg, Schaezlestr. 1, 79104 Freiburg, Germany
² Signalling Research Centres BIOSS and CIBSS, Schaezlestr. 18, 79104 Freiburg, Germany
³ Cluster of Excellence livMats @ FIT – Freiburg Center for Interactive Materials and Bioinspired Technologies, University of Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany

Corresponding author: Decker, Eva L (eva.decker@biologie.uni-freiburg.de)

Introduction
Mosses are an inconspicuous group of plants, which are perceived by people in extremely different fashions: While being an indispensable element of Japanese gardens, they are combatted in Western-European lawns, but due to their small size, are neglected by most. Mosses, however, which belong to the evolutionary oldest land plant group, the bryophytes, developed lifestyles and strategies making them attractive for biotechnological exploitation. While vascular plants resist desiccation by having protective layers of structural biopolymers (cutin, suberin and lignin) and in parallel allow gas exchange via stomata, mosses are dependent on wet environments for active growth. They comprise mainly tissues one-cell layer thick lacking stomata in the photosynthetically active leafy parts of the plants. Mosses develop stomata, dependent on the action of the bHLH transcription factors SCRM and SMF1, exclusively in the sporocarp, the sporophytic generation of the plant [1]. The formation of a phenol-enriched cuticle in the moss Physcomitrella patens only starts with the development of the adult plant, the gametophore. It provides some protection against dehydration as well as stiffness to allow erect growth and it displays commonalities shared among cutin, suberin, lignin and sporopollenin polymers of tracheophytes [²,³]. However, the poikilohydric lifestyle of mosses (i.e. the water content of the cells is equivalent to that of their environment), required the establishment of biochemical mechanisms to cope with abiotic stress, for example drought, which are valuable for future biotech applications in crop plants [4]. Moreover, lacking true roots, mosses take up nutrients via their whole surface, which makes them interesting targets for biomonitoring approaches of environmental pollution. Life in a wet environment in close contact to organisms involved in biodegradation could be the reason for the presence of several special metabolites with antimicrobial or antifungal effects in bryophytes, which have been used widely in traditional medicine [4, ⁵]. The antiseptic and water-absorbing properties of Sphagnum mosses have been used, for example for wound dressing [⁵]. More recently, however, lab-grown P. patens and mixed samples of six moss species taken from the environment were successfully used to create a bryoMFC (microbial fuel cell). The importance of the microbial populations for electron delivery was confirmed [⁶].

The low degree of structural complexity and the predominantly haploid gametophytic growth made mosses interesting objects for cell biology studies [⁷] and genetic-engineering approaches, the latter furthered by the detection of highly efficient gene targeting via homologous recombination in P. patens [⁸]. Homology-directed repair is a means of DNA repair frequent in mosses compared to other plants and all other higher eukaryotes, but it is enhanced in the G2 phase of the cell cycle in vertebrates [⁹]. The G2 phase, however, is the dominant cell-cycle phase within Physcomitrella protonema (the simple juvenile moss tissue which develops from a germinated spore and has filamentous or sometimes thallloid morphology) [¹⁰]. Recently, members of the RecQ helicase family were found to antagonistically influence gene targeting in P. patens [¹¹].

These features, together with an easy cultivation under axenic conditions in various types of devices, including
photobioreactors, also promoted the genetic characterisation by transcriptomics [12,13,14,15] and genome sequencing of initially *P. patens* [16,17], but also the liverwort *Marchantia polymorpha* [18] and peat moss (*Sphagnum* species) [19]. Sequencing of *Physcomitrella* was executed by the U.S. Department of Energy Joint Genome Institute (JGI) as one of seven Plant flagship genomes because of its genetic amenability, the simple morphology, which allows cell biology and developmental studies and its evolutionary position at the base of land plant evolution (https://jgi.doe.gov/why-sequence-physcomitrella-patens/). This extremely good genetic characterisation also largely facilitated biotechnological engineering approaches, for example the successful use of *Physcomitrella* as a bioreactor for metabolite and especially biopharmaceutical production [20]. In the following, we will summarise the recent strategies for moss biotechnological approaches.

**Metabolites from mosses**

The traditional medicinal use of mosses may be based on a multitude of genes, which can be connected to specialised metabolism [5,12]. Consequently, earlier approaches aimed at identifying interesting moss metabolites and deducing the encoding genes to increase the nutritional value of crop plants via genetic engineering [21,22]. Of special importance are the high contents of polyunsaturated and very long-chain fatty acids in several moss species [23].

A very recent use of a biotechnologically produced aqueous cell extract from the moss *P. patens* led to the development of the first moss-based active cosmetic ingredient MossCellTec™ No. 1 by Mibelle Biochemistry. The extract, generated from a wave-bag photobioreactor protonema culture (Figure 1), demonstrated a protective effect on a 3D human reconstituted skin model under climatic stress conditions [24]. It improved the expression of nuclear envelope and transport genes in epidermal keratinocyte cells derived from an older donor, thus leading to the cosmetics concept of cell nuclear health [25].

In addition, *Physcomitrella* demonstrated its feasibility for metabolic engineering, that is, as a production host for commercially valuable metabolites, among those diterpenoids as taxadiene and sesquiterpenoids like the fragrance patchouliol or the anti-malaria drug artemisinin [20,26,27]. From the fragrance-producing mosses [27], the product Fragrant Moss was developed as natural air freshener, and launched by Mosspiration Biotech in January 2019 (https://www.mosspirationbiotech.com/fragrant-moss). A *Physcomitrella* line lacking endogenous diterpenoids due to targeted knockout of the single existing bifunctional diterpene synthase gene [28] was used to produce three different diterpenes from flowering plants, suggesting moss as production host for industrially relevant diterpene biomaterials [29]. As metabolite formation often employs multigenic biosynthesis pathways, the establishment of an efficient protocol for *in planta* self-assembly of several linear DNA building blocks via

![Figure 1](https://www.sciencedirect.com)

**Cultivation of *Physcomitrella patens* for biotechnological processes.** (a) The filamentous juvenile tissue, protonema allows standardised cultivation conditions (scalebar 50 μm). (b) Stirred tank photobioreactor for small-scale production. (c) GMP-production facility. (d) 100 L wave-bag photobioreactor for industrial applications. Photographs credit to Nelly Horst (a), Melanie Heck (b), (c + d) taken from Ref. [38] with permission.
homologous recombination [30] may further promote the industrial use of Physcomitrella as an engineering platform.

**Moss-based biopharmaceuticals**

As high-value pharmaceutical products, recombinant pharmaceutical proteins display an increasing sector within the pharmaceutical market [31]. In contrast, there is still only one plant-made pharmaceutical which has received market release. Eelyso, a β-glucocerebrosidase developed by Protalix and marketed by Pfizer as an enzyme-replacement therapy for Morbus Gaucher is produced in carrot cell cultures and was released by the FDA in 2012. More recently, ZMapp from Nicotiana benthamiana-based production was administered to patients infected with the Ebola virus during the Ebola epidemic in West Africa in 2014 [32]. The first plant-derived vaccine, haemagglutinin virus-like particles against influenza, which was developed by Medicago Inc., reached phase 3 clinical trials [33].

In addition to these plant-based systems *P. patens* has proven itself as a host to be reckoned with for plant-derived biopharmaceutical production. The excellent genetic amenability helped to prevent the attachment of plant-specific O-linked glycans to proteins of interest [34] as well as to humanise the *Physcomitrella* protein N-glycosylation pattern via precise gene targeting [35,36]. The host-cell proteome in the medium, to which products are secreted, is identified, and standardised photobioreactor cultivation processes with a scale up to 500 L are developed [37,38] (Figure 1). These factors triggered the synthesis of several recombinant glycoproteins in this system [38,39**,40*]. The first product, Greenovation’s moss-aGal, successfully passed clinical phase I at the end of 2017 [41]. This enzyme is being considered as an enzyme replacement therapy (ERT) for patients suffering from deficient or defective α-galactosidase A resulting in the lysosomal storage disease Morbus Fabry. A single dose of moss-aGal was tolerated by the patients without any severe side effects and decreased the levels of the substrate globotriaosylceramide (Gb3) even after 28 days [42**].

The parental line used to produce the glycoprotein moss-aGal [43] was a line in which posttranslational Asparagine (Asn, N)-linked protein glycosylation had been engineered to be free of plant-specific sugar residues. This was accomplished by targeted knockout of a β1,2-xylanosyltransferase (XT) and an α1,3-fucosyltransferase (FT) gene [44] to avoid any adverse side effects during patients’ treatments.

While alternative products are available on the market to fight Fabry disease, the glyco-engineered Δxt/ft line was also the basis for the production of two completely new pharmaceuticals, moss-derived human factor H (moss-FH) and the synthetic FH-related multitarget protein MFHR1. Both proteins may have regulatory functions in the human complement system, an important part of the so-called innate immunity [39**,40*,45,46]. Factor H is the main regulator of the alternative pathway of complement activation and mutations affecting FH could lead to severe kidney and eye diseases, among those the atypical haemolytic uremic syndrome (aHUS) and C3 glomerulopathies (C3G) as well as age-related macular degeneration (AMD), the main cause for loss of central vision in the elder population. In pre-clinical studies moss-FH reduced disease indicators in a FH-deficient mouse model bearing C3G symptoms, thereby suggesting itself as the first physiologic therapeutic for patients suffering from complement-related disorders [39**]. MFHR1 is a synthetic protein comprising domains of FH and the related protein FH-related 1 thus being able to target dysfunctions on all levels of complement activation [40*]. Current treatment of FH-related complement disorders are by plasmapheresis or the monoclonal antibody Eculizumbab, currently the most expensive biopharmaceutical protein product, which, however, is only partially effective for esp. C3G patients [47]. Thus, the moss-FH related products may become well tolerated, cost-efficient complement therapeutics [39**,40*].

**Moss for biomimetics**

Recently, mosses from the Funariaceae family (*P. patens* and related species) have been employed in inspiring architectural design or the development of new biomaterials from moss research. In an interdisciplinary collaboration of architects and biologists the genomic relationships of different mosses will be computed to derive algorithms for evolutionary processes that could be applied for exploratory (architectural) design approaches [48]. A second collaborative initiative aimed at analysing structural and functional features of FtsZ networks, which display polymeric structures within chloroplasts which form the plastoskeleton in *Physcomitrella* [49,50]. 3D confocal laser scanning microscopy was used for the acquisition of the networks built by different fluorescence-labelled FtsZ isoforms. From these a computational framework was developed to classify the different protein network structures. With the help of machine learning, features could be extracted that allowed a classification to distinguish between two FtsZ isoforms [49,51]. By linking network structure and dynamics with functionality, the design of protein-engineered biomaterials for regenerative medicine is a vision for future applications [52].

**Biomonitoring and Sphagnum farming**

For several years, various moss species have been used for biomonitoring approaches to measure air quality. Mosses are particularly suitable as they take up elements and nutrients via their surface directly from atmospheric deposition thus reflecting the chemical composition of their environment [53]. Among four frequently used moss genera the genus *Sphagnum* was suggested for superior uptake of heavy metal ions [53]. Biomonitoring is performed with the so-called moss-bag technique, the
exposition of moss material in inert mesh bags at different sites of interest [54]. To provide standardised and comparable raw material for the measurements, however, transplants from the environment are not suitable and in the case of Sphagnum, peat mosses, were unacceptable due to their environmental protection, for example by the European Council Habitats Directive (92/43/EEC). A breakthrough towards standardisation and high biomass increase was the development of an axenic in vitro-cultivation process in 5 L and 12 L-tank photobioreactors [55] (Figure 2). Biomass increased around 30-fold within 4 weeks of bioreactor cultivation of the clonal gametophore material. Process parameters included were mechanical stress, inoculum density, medium compounds and pH [55]. This material was used to monitor atmospheric metal pollutants in lab experiments [56] and for the first time, the uptake of polystyrene nanoparticles was demonstrated, offering the possibility for monitoring microplastics in fresh water environments with the biotechnologically produced peat moss [57]. In a comparative field trial, covering different environmental areas of Italy and Spain, bioreactor-produced S. palustris was superior to native Pseudoscleropodium purum in terms of metal uptake and accumulation [58]. These studies were performed with the ‘mosspine’, a passive contaminant-sampling device superior to former techniques and invented by the EU-funded MOSSclone consortium (www.mossclone.eu) [59]. This device contains devitalised moss within the space of a hollow inner and outer sphere from perforated nylon and plastic material, assuring free air passage and homogenous distribution of the moss. The further improvement of vegetative Sphagnum proliferation for biotechnological use or physiological and cellular interventions might be facilitated by a recently developed method of Sphagnum protonema growth [60].

In addition to monitoring environmental pollution, peat mosses contribute to a large extent to global climate. Peatlands, comprising fossil peat mosses, display a tremendously important carbon storage. Covering only 0.5% of the overall land surface, their drainage by harvest of peat for the horticultural industry or for their use as, for example farmland or grassland, contributes with 32% to the global cropland greenhouse gas (GHG) emissions [61,62]. ‘Sphagnum farming’ – the production of Sphagnum biomass on rewetted peatlands – will help to halt GHG emissions from drained peatlands, thus meeting urgent political objectives to reduce CO2 emissions. Additionally, Sphagnum farming is meant to provide a sustainable source for peat moss-biomass as an effective replacement of peat, which still displays the best-quality potting soil growth medium by far [61]. While Sphagnum is propagating slowly under natural conditions, the axenic large-scale production in photobioreactors will speed up the production of starting material drastically (Figure 2). Setting up large-scale production processes is one of the aims of the MOOSZucht initiative funded by the German Federal Ministry of Nutrition and Agriculture in 2017 (https://www.moorwissen.de/en/paludikultur/projekte/torfooskultivierung/mooszucht.php).

Conclusions
These small plants, mosses, have made their way from use in traditional medicine to a manifold of uses today: They please consumer affections with a genetically engineered fragrant moss and the first bioactive ingredient from a moss for the cosmetic industry on the market. They also provide opportunities for treating severe human diseases and the first moss-made drug candidate successfully passed clinical trial phase I. Peat-moss
The authors are inventors of patents and patent applications related to topics discussed here. RR is an inventor of the moss bioreactor and a founder of Greenovation Biotech. He currently serves as advisory board member of this company.

Acknowledgements

We acknowledge current funding by the German Research Foundation (DFG) under Germany’s Excellence Strategy (CIBSS – EXC-2189 and livMatS – EXC-2193/1 – 2100247729), the German Federal Ministry of Food and Agriculture (BMLF 220072/16), and by the Marie Curie Actions of the European Union’s Horizon 2020 (Grant agreement no. 765115 MossTech). We are indebted to Anne Katrin Prowse for proof-reading of the manuscript.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

+ of special interest
+ of outstanding interest

18. The much-improved genome assembly and annotation reveals no distinct centromeres in Physcomitrella, unusual DNA-methylation patterns and remnants from an ancient integration of a giant virus into the moss genome.
The paper reports the first full pathway engineering in Physcomitrella. The expression of five pathway enzymes shows promising results for artemisinin production and in future additional specialised metabolites in moss.


29. Banerjee A, Arnesen JA, Moser D, Motsa BB, Johnson SR, • Hamberger B: Engineering modular diterpene biosynthetic pathways in *Physcomitrella patens*. *Plants* 2019, 249:221-233. The study sorts the opportunities for production of industrially relevant diterpene biomaterials in moss. To avoid any formation of moss-derived by-products, a disruption line was used serving as platform line devoid of endogenous diterpene metabolites.


42. Hennermann JB, Arash-Kaps L, Fekete G, Schaaf A, Busch A, • Frischmuth T: Pharmacokinetics, pharmacodynamics, and safety of moss-α-galactosidase A in patients with Fabry disease. *J Inherit Metab Dis* 2019, 42:527-533 http://dx.doi.org/10.1002/jimd.12052. The study presents the results of the first clinical trial phase I for a moss-produced biopharmaceutical. The product was well tolerated by all patients and decreased the concentration of disease markers in body fluids.


The study successfully employs the newly invented mossphere and demonstrates that bioreactor-produced Sphagnum is superior to field-grown mosses in an air pollution-monitoring approach.


