



Tansley review

How to build a lichen: from metabolite release to symbiotic interplay

Author of correspondence:
Ilse Kranner
Email: ilse.kranner@uibk.ac.at

Received: 25 October 2022
Accepted: 28 December 2022

Gregor Pichler¹ , Lucia Muggia² , Fabio Candotto Carniel² ,
Martin Grube³ and Ilse Kranner¹

¹Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria; ²Department of Life Sciences, University of Trieste, Via L. Giorgieri 10, 34127 Trieste, Italy; ³Institute of Biology, University of Graz, Holteigasse 6, 8010 Graz, Austria

Contents

Summary	1362	IV. The thallus: a fine-tuned symbiotic interplay supporting nutrition, tolerance of irradiation and desiccation, and reproduction	1372
I. Introduction: from a 'dual nature of lichens' to a comprehensive one	1362	V. Outlook	1374
II. Lichenization: transition from a free-living to a symbiotic state	1365	Acknowledgements	1374
III. From arabitinol to zeatin: a gradual shift in the contribution of metabolites from beginning to completion of lichenization?	1366	References	1375

New Phytologist (2023) **238**: 1362–1378
doi: 10.1111/nph.18780

Key words: extracellular, fungus, metabolite, mycobiont, photobiot, polyol, signalling, symbiosis.

Summary

Exposing their vegetative bodies to the light, lichens are outstanding amongst other fungal symbioses. Not requiring a pre-established host, 'lichenized fungi' build an entirely new structure together with microbial photosynthetic partners that neither can form alone. The signals involved in the transition of a fungus and a compatible photosynthetic partner from a free-living to a symbiotic state culminating in thallus formation, termed 'lichenization', and in the maintenance of the symbiosis, are poorly understood. Here, we synthesise the puzzle pieces of the scarce knowledge available into an updated concept of signalling involved in lichenization, comprising five main stages: (1) the 'pre-contact stage', (2) the 'contact stage', (3) 'envelopment' of algal cells by the fungus, (4) their 'incorporation' into a pre-thallus and (5) 'differentiation' into a complex thallus. Considering the involvement of extracellularly released metabolites in each phase, we propose that compounds such as fungal lectins and algal cyclic peptides elicit early contact between the symbionts-to-be, whereas phytohormone signalling, antioxidant protection and carbon exchange through sugars and sugar alcohols are of continued importance throughout all stages. In the fully formed lichen thallus, secondary lichen metabolites and mineral nutrition are suggested to stabilize the functionalities of the thallus, including the associated microbiota.

'The whole is more than the sum of its parts'

Attributed to Aristotle

I. Introduction: from a 'dual nature of lichens' to a comprehensive one

Mutualistic or self-sustaining symbioses usually emerge in evolution through selection of novel pathways or phenotypes

(Douglas, 2014). Indeed, the symbiotic stage often looks completely different from what the symbiotic partners produce in axenic solitude. This is particularly so with lichens, where the knitwork of fungal hyphae produces a unique miniature glasshouse to grow algae – a structure that surpasses most other vegetative fungal mycelia by the complexity of its organisation. Unsurprisingly, lichens were the first discovered case of symbioses, after they

were considered as an own lower plant-like group with unclear affiliation, although they were probably not the first to reach dry grounds in evolutionary history (Nelsen *et al.*, 2020). Five-hundred to 450 million years ago (Ma), the colonization of terrestrial habitats – when the descendants of streptophyte algae evolved into the first land plants – was a major transition on Earth (Becker, 2013), and the ability of plants to form symbiotic associations with beneficial, mycorrhizal fungi was one of the critical innovations required (Delaux *et al.*, 2015). With the first undisputed appearance of lichens in the Lower Devonian, *c.* 415 Ma (Selosse & Le Tacon, 1998; Honegger *et al.*, 2013), the lichen symbiosis could be viewed as a second, alternative way of conquering the land, when the Chlorophyta, together with the Ascomycota, evolved the first lichen thalli. Since then, lichens have massively diversified (Selosse & Le Tacon, 1998; Selosse *et al.*, 2015), and their symbiotic lifestyle seems to be a requirement for sexual reproduction of the involved fungus, also implying that the signalling framework required to synthesize the symbiotic state is likewise conserved.

The ‘dual nature’ of lichens, first described by Schwendener, was initially interpreted as controlled parasitism, where an alga is ‘enslaved by a fungus and compelled into its service’ (Schwendener, 1869), and first attempts to culture them *in vitro* started soon after (Bornet, 1872). Nowadays, lichens are regarded as self-sustaining ecosystems formed by the interaction of an exhibitant fungus, called ‘mycobiont’, forming a symbiosis with extracellular microbial photosynthetic partners, termed ‘photobiont’, and an indeterminate number of other microscopic organisms (Hawksworth & Grube, 2020). About 19 400 mycobiont species in 115

families have been identified, mostly Ascomycota, and a growing number of Basidiomycota species as their main fungal symbionts (Lücking *et al.*, 2016). Considerably fewer species (*c.* 120) of unicellular green algae in the Chlorophyta are the known photobionts of an estimated 85% of lichens. Cyanobacteria are photobionts of another 10% of lichens. Around 3% are tripartite symbioses, in which both green algae (‘chlorobionts’) and cyanobacteria (‘cyanobionts’) co-exist, whereby the cyanobacteria are usually compartmented in the so-called ‘cephalodia’ within the thallus, and some lichen photobionts are yet to be described (Honegger, 2012). Furthermore, ‘lichen parasites’ or ‘lichenicolous fungi’ are known as specific fungal colonizers of lichen thalli, some of which – the ‘lichenicolous lichens’ – even form their own thallus structures (Lawrey & Diederich, 2003). Recent research illuminated the diversity of other micro-organisms, that is the lichen microbiota (Grube & Berg, 2009; Muggia *et al.*, 2016; Spribille *et al.*, 2016; Spribille, 2018). Nevertheless, the mycobiont constitutes the main proportion of lichen biomass and, with very few exceptions, builds up the structures of the lichen thallus, shaping the various growth forms (Fig. 1) of lichens.

The primary lichen myco- and photobionts can reproduce vegetatively by tiny structures containing the lichen-forming fungi and their photobionts present in the parental thallus, but free-living stages of the prospective symbionts are also found in nature (Tscheramak-Woess, 1978; Bubrick *et al.*, 1984). Fungal dispersal units may be developed meiotically or mitotically. As no active structures for algal dispersal are known to occur in lichen thalli, photobionts are thought to be released from an established thallus, for example due to mechanical disruption, fungal decay or passage

Fig. 1 Lichen thallus growth forms. (a) *Collema crispum* (gelatinose, foliose/shrubby), (b) *Thamnolia vermicularis* (fruticose, with wormlike branches), (c) *Ramalina fraxinea* (fruticose/shrubby, hanging), (d) *Seiophora villosa* (fruticose/shrubby), (e) *Xanthoria parietina* (foliose); (f) *Lecanora rupicola* (crustose, epilithic) infected by the lichenicolous lichen *Rimularia insularis* (brown patches encircled by yellow dashed lines; yellow arrows), (g) *Cystocoleus ebeneus* (filamentous; arrows indicating the melanised fungus enwrapping *Trentepohlia* sp. filaments), (h) *Cladonia verticillata* (fruticose, arrows indicating the squamulose primary thallus (PT) and the erect podetia of the secondary thallus (ST)). Bars: (a, d, e, h) 1 cm; (b, c) 2 cm; (f) 10 cm; (g) 0.5 mm.



through feeding animals (Peksa *et al.*, 2022). The synthesis of a lichen thallus from a free-living state of compatible fungi and algae (cyanobacteria) has been referred to as 'lichenization' or 're-lichenization' (Sanders & Lücking, 2002), an event infrequently gained and also lost in the Ascomycota (Lutzoni *et al.*, 2001). Alternatively, and instead of synonymous use, the term lichenization could be reserved for the very first transition of a naïve free-living fungus and its future microbial photobionts, through evolution, into a self-sustaining thallus, and re-lichenization to cases in which mycobionts and photobionts were released or separated from existing lichen thalli, either in nature or in the

laboratory, and then re-synthesised into a thallus. Furthermore, perhaps more frequently than expected, free-living lichen-forming fungi may capture compatible green algae from various stages of already existing lichen thalli, and lichenicolous lichens appear to specialize on such photobiont stealing. We suggest the term 'trans-lichenization' to describe the capture of already lichenized algae by another mycobiont, and the newly formed thallus as 'kleptosymbiosis' (Fig. 2). These cases merit differentiating, as previously lichenized algae already experienced transcriptomic (re)-programming, as deduced from the changing ultrastructural details (De Los *et al.*, 2002).

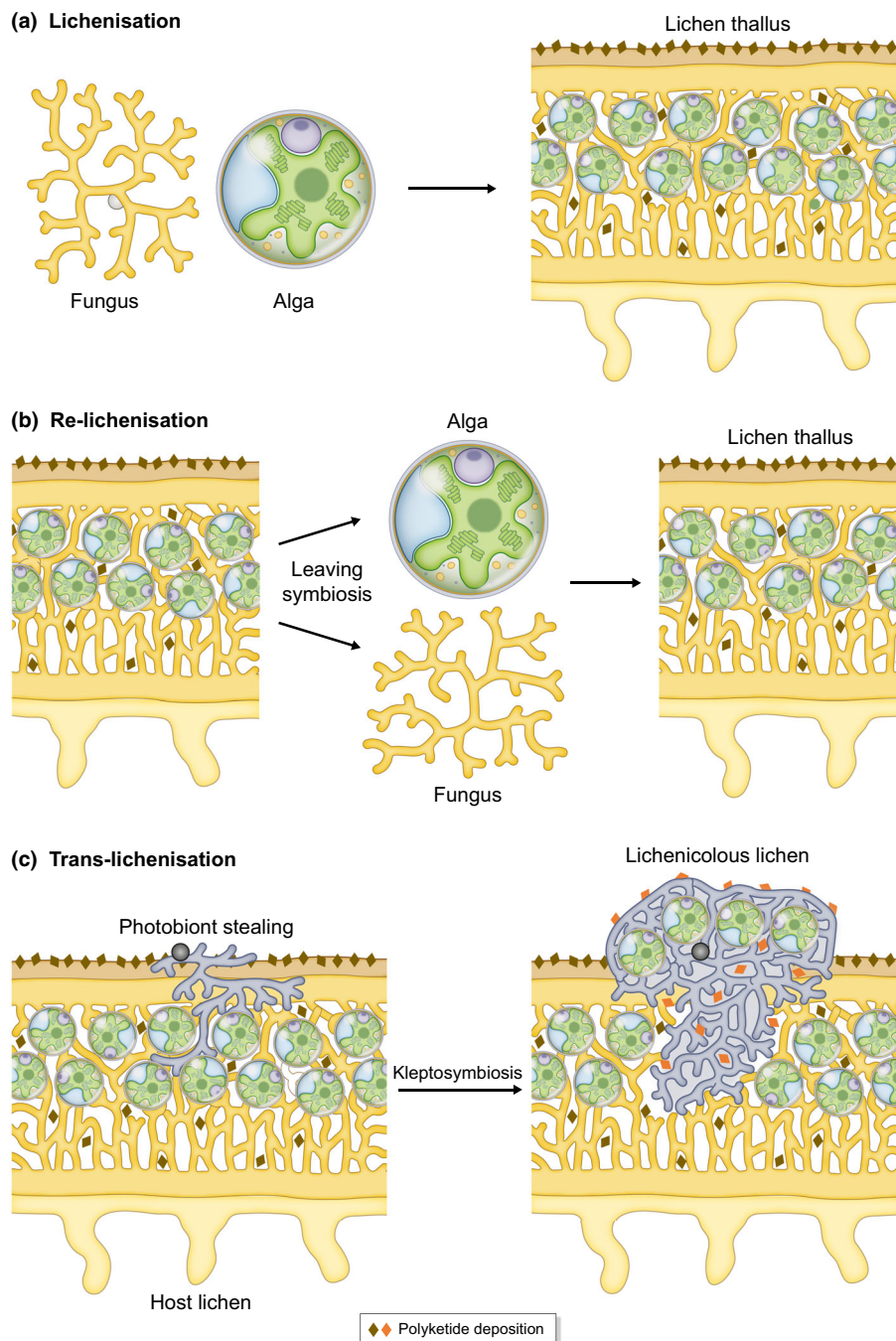


Fig. 2 Proposed concept of lichenization, re-lichenization and trans-lichenization. (a) Lichenization – at an evolutionary time scale – defined as the transition of a free-living fungus and its future microbial photobionts, green microalgae and/or cyanobacteria, culminates in the formation of a lichen thallus, a self-sustaining symbiosis that eventually comprises a plethora of other micro-organisms in the lichen microbiota. (b) Re-lichenization is referred to cases, in which the primary symbionts leave the symbiosis followed by building a new thallus. This occurs in nature, and can be achieved experimentally under laboratory conditions, from which most of our current knowledge on lichenization has been obtained. (c) Trans-lichenization is shown for, although not restricted to, lichenicolous lichens, which capture photobionts from other lichens. This photobiont stealing by lichen-forming fungi and establishment of yet another lichen thallus is proposed to be termed kleptosymbiosis.

The specific processes involved in re-programming through trans-lichenization may explain why lichenicolous lichens are generally very selective for their hosts. In addition, there are also some examples of 'lichen evolution in progress'. For example, *Schizoxylon* sp. can either live alone, saprophytically, or form a loose symbiosis with poorly distinguishable thalli together with *Coccomyxa* sp. photobionts (Muggia *et al.*, 2011), and some of the filamentous *Trentepohlia* species can occur in free-living colonies, be lichen photobionts (Fig. 1g) or parasites (Hametner *et al.*, 2014). Most knowledge gained about lichenization is derived from laboratory studies, that is re-lichenization *sensu stricto*, but below we continue to use the terms lichenization and re-lichenization synonymously, in line with the literature available.

II. Lichenization: transition from a free-living to a symbiotic state

1. The stages of lichenization

A synthesis of topical papers (Trembley *et al.*, 2002; Joneson & Lutzoni, 2009; Joneson *et al.*, 2011; Meeßen *et al.*, 2013; Meeßen & Ott, 2013; Athukorala *et al.*, 2014; Insarova & Blagoveshchenskaya, 2016; Kono *et al.*, 2020) on lichenization of fruticose or squamulose lichens, mostly in the orders Lecanorales or Teloschistales, reveals that lichenization is currently thought to comprise several steps. These steps are summarized in Fig. 3 and envisaged to blend smoothly from one into the other, that is without abrupt shifts. In the 'pre-contact stage', the fungus and alga are in close proximity, but without physical contact. It is assumed that chemical signalling supports mutual recognition in this phase, followed by increased mucilage production and hyphal branching of the fungus and growth towards the algae. This first, visually detectable indication of a mycobiont recognizing a potential photobiont is followed by the 'contact stage', during which mycobiont hyphae establish physical contact with photobiont cells, typically through appressoria or different types of haustoria (Honegger, 1986, 2012). In the 'envelopment stage', the fungal hyphae enwrap the photobiont cells almost completely. In the 'incorporation stage', hyphae and photobiont cells form an undifferentiated aggregation of biomass, and the 'differentiation stage' entails organization into a (commonly stratified) thallus, which can only be achieved by compatible myco- and photobionts (Meeßen & Ott, 2013). In the fully formed thallus, the mycobiont controls the development of photobiont cell division and growth, as well as regulating photobiont cell size and ultrastructure (Hill, 1989, 1993). It is likely that this control by the fungus commences early during lichenization.

It is not precisely known how long it takes to complete lichenization after the first sensing among the partners in nature, but on exposed plastic leaves in a rain-forest lichen symbioses established within relatively short periods of time, in the range of months (Sanders, 2002). In the laboratory, at least a few months up to several years of co-culture of previously isolated primary symbionts are required to achieve re-lichenization into fully formed lichen thalli (e.g. Stocker-Wörgötter & Türk, 1991;

Yoshimura *et al.*, 1993; Stocker-Wörgötter, 2001a,b; Kono *et al.*, 2020). Specific abiotic factors such as cycles of desiccation and rehydration promote *in vitro* lichenization (Stocker-Wörgötter, 2001b). A need for desiccation also agrees with the suggestion that the ability to tolerate desiccation and extensive irradiation is a prerequisite for, and driving, lichen evolution (Kranter & Lutzoni, 1999).

2. Molecular crosstalk accompanies mutual recognition of the prospective symbionts

Ahmadjian & Jacobs (1981) put forward that a fungus recognizes a prospective photobiont by the shape of the algal cells, referred to as 'thigmotropism hypothesis'. Joneson & Lutzoni (2009) experimentally achieved mycobiont differentiation only with compatible lichen-forming algae rather than inanimate objects such as glass beads. These results, together with the finding that between 11 and 28% of mycobiont and photobiont genes are already upregulated during the 'pre-contact' and the 'contact stage', supported the 'signalling hypothesis' based on chemical signalling between symbionts before physical contact (Joneson *et al.*, 2011). We now know that lichenization is accompanied by regulation of gene expression in each partner involved, and this will induce downstream proteomic and metabolomic re-arrangements (Trembley *et al.*, 2002; Joneson *et al.*, 2011; Armaleo *et al.*, 2019; Kono *et al.*, 2020; Sveshnikova & Piercey-Normore, 2021). For example, genes encoding signal transduction components are already upregulated in the contact stage in both, mycobiont and photobiont, as elegantly demonstrated for the model lichen, *Cladonia grayi*, and its *Asterochloris glomerata* photobiont: specifically, genes encoding extracellular hydrolases and membrane transport proteins are upregulated in both prospective symbionts, and ammonium and ribitol transporters in the fungus only (Armaleo *et al.*, 2019). Furthermore, the microbiota may influence lichen metabolism, for example phytohormone, vitamin, carbohydrate, lipid and nitrogen metabolism, thereby contributing to the fine-tuning of the symbiotic relationship of lichens, especially when challenged by biotic and abiotic stress factors (Erlacher *et al.*, 2015; Cernava *et al.*, 2017). Considering these transcriptional responses, symbiotic interaction must be accompanied by molecular crosstalk, that is the intricate process by which signal transduction pathways are affected by components of others. Importantly, crosstalk requires chemical compounds to be released into the extracellular matrix and perceived by the symbiotic partner. Signals can then be processed within the cell to induce downstream effects on the transcriptome, proteome and metabolome, and thus physiology and morphology of the symbiotic partners. However, our knowledge of compounds that affect signal transduction pathways during lichenization is still in its infancy. Next, we review reports on compounds released by lichen mycobionts and photobionts into the extracellular space (see Fig. 4 for overview), addressing potential roles of primary and secondary metabolites from the early stages of lichenization up to the fully formed thallus, appreciating that all are important for the development of a functional thallus.

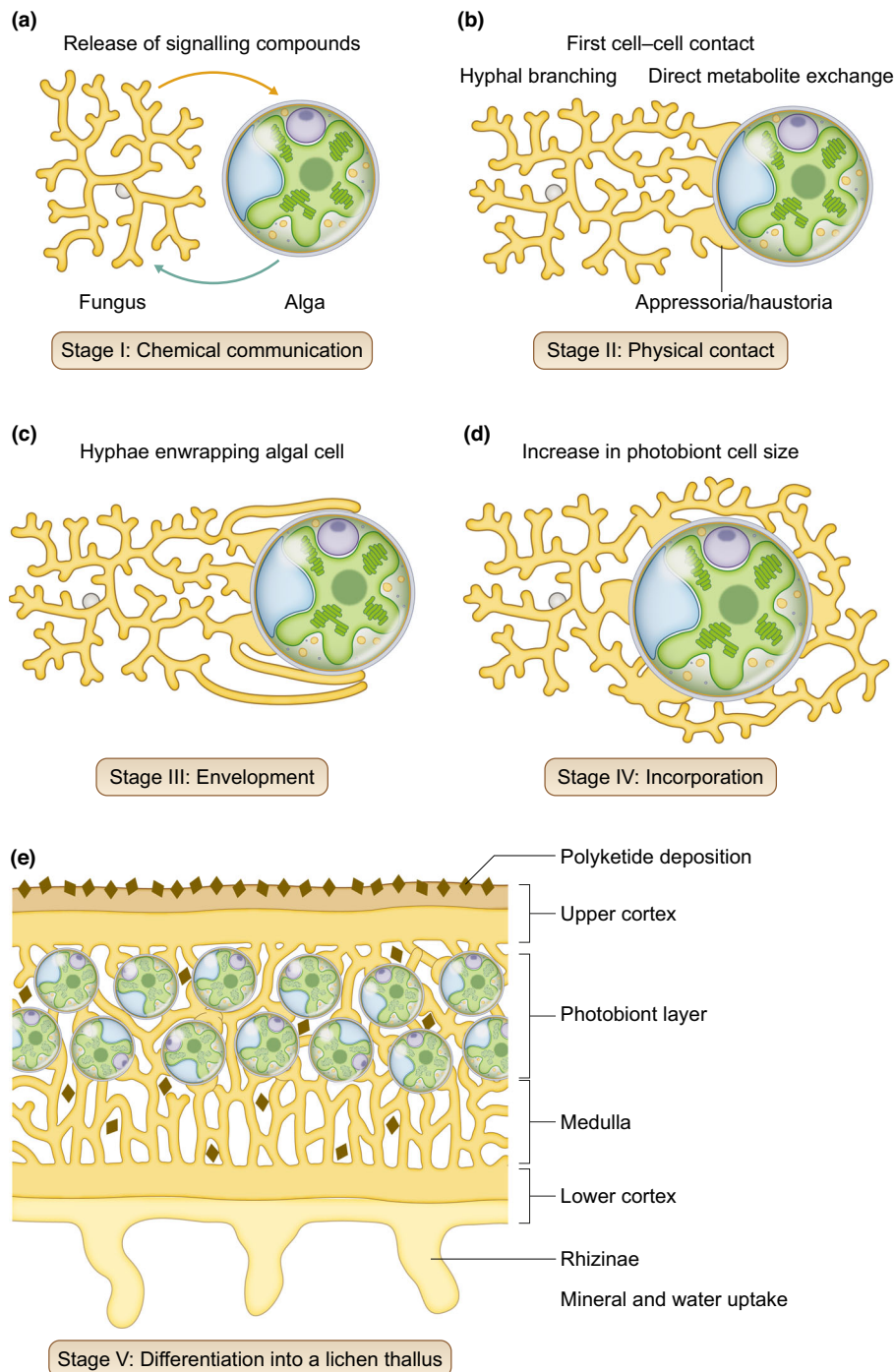


Fig. 3 The stages of lichenization. (a) The 'pre-contact stage', before fungus and alga are in physical contact, is characterized by increased hyphal branching and mucilage production by the fungus, and release of molecules by the fungus and the alga into the environment, supporting mutual recognition. (b) In the 'contact stage', appressoria and haustoria are formed by the fungus, physically connecting mycobiont hyphae and photobiont cells, enabling direct exchange of metabolites. (c) In the 'envelopment stage', fungal biomass increases, more appressoria and haustoria are formed, and hyphae engulf the photobiont cells. (d) In the 'incorporation' stage, fungal hyphae and photobiont cells form undifferentiated aggregations and algal cell size increases. (e) The final 'differentiation stage' comprises the differentiation into a lichen thallus. See section 'II. Lichenization: transition from a free-living to a symbiotic state' for details and citations.

III. From arabitrol to zeatin: a gradual shift in the contribution of metabolites from beginning to completion of lichenization?

1. Elicitors in the pre-contact and contact stage

Making contact depends on prior recognition between the symbionts, and evidence is emerging that elicitors interacting with the cell wall are involved (Wang *et al.*, 2014; Díaz *et al.*, 2016). Lectins, although they are not receptors *per se*, are proteins that bind

to specific carbohydrate residues and mediate attachment of micro-organisms such as fungi, bacteria, microalgae or viruses to target cells (Singh & Walia, 2014; Romero *et al.*, 2021). When lectins recognize their target carbohydrates embedded in the outer surface of cell walls, cells are bound and lectins can activate enzyme cascades involved in signalling processes, inducing metabolic and physiological rearrangements (Molina & Vicente, 1995; Alen'kina *et al.*, 2014). Using the model lichen, *Xanthoria parietina*, Bubrick & Galun (1980) showed that mycobiont proteins can bind to acidic polysaccharide residues on the outer cell wall of five photobiont species isolated from

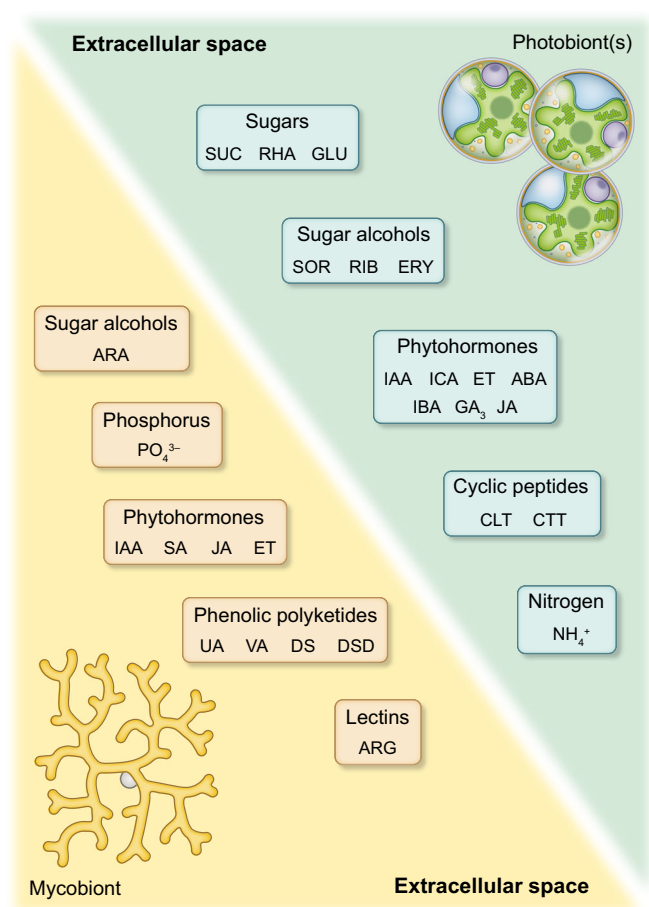


Fig. 4 Chemical compounds released by isolated lichen myco- and photobionts into their environment. Photobionts have been shown to release the sugars glucose (GLU), rhamnose (RHA) and sucrose (SUC), the sugar alcohols sorbitol (SOR), ribitol (RIB), and erythritol (ERY), the phytohormones indole-3-acetic acid (IAA) and its precursor indole-carbaldehyde (ICA), indole-3-butyric acid (IBA), abscisic acid (ABA), gibberellin A3 (GA_3), jasmonic acid (JA), and ethylene (ET), and the cyclic peptides cyclo-(L-leucyl-L-tyrosyl) (CLT), and cyclo-(L-tryptophyl-L-tryptophyl) (CTT). Mycobionts release the sugar alcohol arabinol (ARA), phosphate (PO_4^{3-}), the phytohormones IAA, salicylic acid (SA), JA, and ET, lectins with arginase (ARG) activity, and the phenolic polyketides usnic acid (UA), vulpinic acid (VA), and various depsides (DS), and depsidones (DSD). See text and Tables 1 and 2 for details and references.

Teloschistaceae lichens, but not to photobionts from other lichen families, suggesting that only compatible photobionts are targeted. A fungal lectin with arginase activity binding to polygalactosylated urease was found in the photobiont cell wall, and glycosylated urease production was limited to periods of algal cell division (Sacristán *et al.*, 2006). Arginase secretion emerges as a key factor in the recognition of compatible algae, and fungal lectins appear to be involved in the differentiated recognition of green algae and cyanobacteria, leading to the formation of chlorolichens and cyanolichens, respectively (Legaz *et al.*, 2004; Vivas *et al.*, 2010), even though the precise recognition mechanisms remain to be elucidated. Recruitment of *Nostoc* sp. cells by *Peltigera canina* seems to be induced by glycosylated arginase, the adhesion of which to the hyphal surface lectins is achieved by contraction-relaxation episodes of the cytoskeleton (Díaz *et al.*, 2011).

Moreover, a cyclic peptide, cyclo-(L-tryptophyl-L-tryptophyl) was found in the exudates of four different *Trebouxia* sp. photobionts isolated from the lichens *Gyalolechia bracteata* (syn. *Fulgensia bracteata*), *Gyalolechia fulgens*, *Toninia sedifolia* and *Rusavskia elegans* (syn. *Xanthoria elegans*). *Asterochloris* sp. isolated from *Romularia lurida* (syn. *Lecidea lurida*), released cyclo-(L-leucyl-L-tyrosyl) in addition to cyclo-(L-tryptophyl-L-tryptophyl). When applied exogenously to the *Gyalolechia bracteata* mycobiont, cyclo-(L-tryptophyl-L-tryptophyl) triggered ascospore germination (Meeßen *et al.*, 2013). Therefore, the sparse evidence suggests that in the precontact and the contact stage, fungal lectins – as readers of the sugar code – elicit contact and are involved in recognizing compatible photobionts, and algal metabolites such as cyclic peptides released into the extracellular environment attract potential mycobionts, and affect fungal sexual reproduction.

2. Sugars and sugar alcohols from the precontact stage through to the fully established lichen thallus

Within the intact thallus, and also when isolated and grown axenically, myco- and photobionts release sugars and sugar alcohols into the extracellular space (Table 1). Sugar alcohols containing multiple hydroxyl groups are often referred to as 'polyols', but this term is somewhat ambiguous as it is also used in polymer chemistry, so that we prefer the term sugar alcohols. These molecules appear to be early attractants for the carbohydrate-loving mycobionts *in spe*. Release of sugar alcohols was reported mainly for photobionts, disregarding release by the mycobiont, with one exception (Wang *et al.*, 2014), perhaps reflecting an anticipated role of the photobiont as the main nurturer. When isolated from the thallus and then co-cultivated with its mycobiont, the photobiont *Diplosphaera chodatii* released sorbitol, glucose and sucrose, and the mycobiont incorporated these compounds using specific transporters. Sorbitol and sucrose can be then converted into fructose and glucose, and enter fungal glycolysis. However, in the absence of the mycobiont, sorbitol, glucose and sucrose were not found in exudates of axenically cultured *Diplosphaera chodatii* photobionts, so it appears that a stimulus from the mycobiont is required to trigger the release of these compounds (Wang *et al.*, 2014). If this assumption is correct, it follows that chemical communication between the myco- and photobionts through sugars and sugar alcohols contains push-pull elements, that is transport from sink to source. Fungal stimuli could involve the actions of yet to be determined members of fungal carbohydrate-active enzymes (CAZymes), which may enhance the permeability of algal cell walls. Furthermore, the isolated photobionts of *Gyalolechia bracteata*, but not free-living green algae, also release rhamnose, which may be specific for lichen-forming algae (Meeßen *et al.*, 2013). Interestingly, unlike cyclo-(L-tryptophyl-L-tryptophyl), rhamnose suppresses ascospore germination (Meeßen *et al.*, 2013). Moreover, exogenous treatment with sugars and sugar alcohols affects the growth of cultured lichen mycobionts. For example, ribitol stimulated growth of the *Ramalina farinacea* and *Ramalina fastigiata* mycobionts (Wang *et al.*, 2009), and mannitol and glucose that of the *Xanthoria parietina* mycobiont, although in the latter growth rates decreased with increasing ribitol

Table 1 Sugars and sugar alcohols released by lichen myco- and photobionts.

Mycobiont Photobiont	Sugars	Sugar alcohols	References
Coccocarpia sp.	nd	nd	Richardson <i>et al.</i> (1968)
<i>Scytonema</i> sp.	Glucose*	nd	Hill & Smith (1972)
Collema auriculatum	nd	nd	
<i>Nostoc</i> sp.	Glucose*	nd	Richardson <i>et al.</i> (1968)
Dermatocarpon hepaticum	nd	nd	Richardson <i>et al.</i> (1968)
<i>Myrmecia</i> sp.	nd	Ribitol*	Hill & Smith (1972)
Dermatocarpon miniatum, D. fluviatile	nd	nd	Hill & Smith (1972)
<i>Hyalococcus</i> sp.	nd	Sorbitol*	Richardson <i>et al.</i> (1968)
Endocarpon pusillum	nd	nd	
<i>Diplosphaera chodatii</i>	Glucose, Sucrose	Sorbitol	Wang <i>et al.</i> (2014)
Fulgensia bracteata	nd	nd	
<i>Trebouxia</i> sp.	Rhamnose	nd	Meeßen <i>et al.</i> (2013)
Gyalecta cupularis	nd	nd	
<i>Trentepohlia</i> sp.	nd	Erythritol*	Richardson <i>et al.</i> (1968)
Lecanactis stenhammarii	nd	nd	
<i>Trentepohlia</i> sp.	nd	Erythritol*	Richardson <i>et al.</i> (1968)
Lecanora conizaeoides	nd	nd	
<i>Trebouxia</i> sp.	nd	Ribitol*	Richardson <i>et al.</i> (1968)
Lepraria incana, L. chlorine	nd	nd	
<i>Trebouxia</i> sp.	nd	Ribitol*	Hill & Smith (1972)
Lichina pygmaea	nd	nd	
<i>Calothrix</i> sp.	Glucose*	nd	Richardson <i>et al.</i> (1968)
Lobaria amplissima	nd	nd	
<i>Coccomyxa</i> sp., (<i>Nostoc</i> sp.)	(Glucose)*	Ribitol*	Richardson <i>et al.</i> (1968)
Lobaria laetevirens	nd	nd	
<i>Myrmecia</i> sp.	nd	Ribitol*	Richardson <i>et al.</i> (1968)
Lobaria scrobiculata	nd	nd	
<i>Nostoc</i> sp.	Glucose*	nd	Richardson <i>et al.</i> (1968)
Peltigera polydactyla, P. canina	nd	nd	Drew & Smith (1967)
P. horizontalis, P. polydactyla	nd	nd	Richardson <i>et al.</i> (1968)
<i>Nostoc</i> sp.	Glucose*	nd	Hill & Smith (1972)
Peltigera aphthosa	nd	nd	Hill & Smith (1972)
<i>Coccomyxa</i> sp., (<i>Nostoc</i> sp.)	(Glucose)*	Ribitol*	Richardson <i>et al.</i> (1968)
Pseudevernia furfuracea, P. saxatilis	nd	nd	
<i>Trebouxia</i> sp.	nd	Ribitol*	Richardson <i>et al.</i> (1968)
Porina lectissima	nd	nd	
<i>Trentepohlia</i> sp.	nd	Erythritol*	Hill & Smith (1972)
Ramalina crassa, R. subbreviscula	nd	nd	
<i>Trebouxia</i> sp.	nd	Ribitol*	Komiya & Shibata (1971)
Ramalina yasudae	nd	Arabitol	Kosugi <i>et al.</i> (2013)
<i>Trebouxia</i> sp.	nd	Ribitol	
Rocella montagnei, R. fuciformis, R. phycopsis	nd	nd	Hill & Smith (1972)
<i>Trentepohlia</i> sp.	nd	Erythritol*	Richardson <i>et al.</i> (1968)
Solorina saccata	nd	nd	
<i>Coccomyxa</i> sp., (<i>Nostoc</i> sp.)	(Glucose)*	Ribitol*	Richardson <i>et al.</i> (1968)
Sphaerophorus globosus	nd	nd	
<i>Trebouxia</i> sp.	nd	Ribitol*	Hill & Smith (1972)
Sticta fuliginosa	nd	nd	
<i>Nostoc</i> sp.	Glucose*	nd	Richardson <i>et al.</i> (1968)
Umbilicaria pustulata	nd	nd	
<i>Trebouxia</i> sp.	nd	Ribitol*	Richardson <i>et al.</i> (1968)
Xanthoria aureola, X. calcicola	nd	nd	Richardson <i>et al.</i> (1968)
<i>Trebouxia</i> sp.	nd	Ribitol*	Hill & Smith (1972)
			Lines <i>et al.</i> (1989)

Sugars and sugar alcohols exchanged between lichen symbionts within the thallus assessed using radioactive labelled CO₂ are marked with an asterisk, the rest were detected in exudates of isolated lichen myco- and photobionts. The third photosynthetic partners of tripartite lichens are listed in brackets; nd, not determined. Bold and regular text in the left column indicates mycobiont and photobiont species, respectively.

concentrations (Pichler *et al.*, 2021). Thus, the preference for specific sugars and sugar alcohols as carbon sources may depend on mycobiont species, and sugars and sugar alcohols can affect mycobiont growth in a concentration-dependent manner (Fig. 5). Furthermore, ribitol also suppressed and mannitol enhanced mycobiont secondary metabolite production in axenic mycobiont cultures of *Rusavskia elegans* (Brunauer *et al.*, 2007). Radioactively labelled [^3H] mannitol can be incorporated into the cell walls of isolated mycobionts, but not into the cell walls of other, free-living fungi (Galun *et al.*, 1976). This points to the existence of specific mannitol and other sugar alcohol transporters in lichen mycobionts (Yoshino *et al.*, 2019), but it remains to be tested whether mannitol incorporation into cell walls is a key feature required for lichenization. Based on the finding that many genes encoding CAZymes were found in 46 representative mycobiont genomes, it was suggested, albeit not experimentally proven, that mycobionts could metabolize algal carbohydrates and cell wall components, or carbon from other sources (Resl *et al.*, 2022).

It follows that sugars and sugar alcohols released extracellularly by algae could be incorporated by fungi in the precontact stage, serving as attractants for future mycobionts. So far, no reports are available on the release, uptake or the effects of sugars and sugar alcohols on the fungal and algal transcriptomes during algal envelopment and incorporation by the fungus (stages III and IV in Fig. 3). Nevertheless, sugars and sugar alcohols are key carbon sources exchanged between the symbionts in intact lichen thalli,

and therefore are likely pivotal for the symbiotic interplay between lichen photo- and mycobionts throughout lichenization.

In fully formed lichen thalli, sugars and sugar alcohols produced by photobionts are the main carbon sources for their mycobionts, as demonstrated by pioneering studies from the 1960s and 1970s. For example, ribitol, erythritol, sorbitol and glucose released by lichen photobionts are taken up by their corresponding mycobionts in many lichens (Richardson *et al.*, 1968; Lines *et al.*, 1989). The type of sugars and sugar alcohols provided by lichen photobionts to their mycobionts seems to reflect the phylogenetic status of the photobiont species. Early key studies revealed that glucose is the main photosynthate provided by *Nostoc* sp., ribitol by *Trebouxia*, and erythritol by *Trentepohlia* (Richardson *et al.*, 1968; Hill & Smith, 1972). The current view is that glucose is the main photosynthate transported to fungal mycobionts by cyanobacterial photobionts, whereas the green lineage uses sugar alcohols. Specifically, tetritols, such as erythritol, appear to be the main sugar alcohols shunted to the fungal mycobionts by green microalgae in the Chlorophyceae and Trentepohliales, whereas other clades, including Prasiolales and Trebouxiiales, use pentitols, such as ribitol (see Spribille *et al.*, 2022; for summary).

Intriguingly, mycobionts can convert sugars and sugar alcohols produced by photobionts into other sugar alcohols (Eisenreich *et al.*, 2011) and can even return them to their photobionts. For example, the *Xanthoria calcicola* mycobiont converts ribitol into mannitol, elegantly shown via ^{13}C NMR analysis (Lines

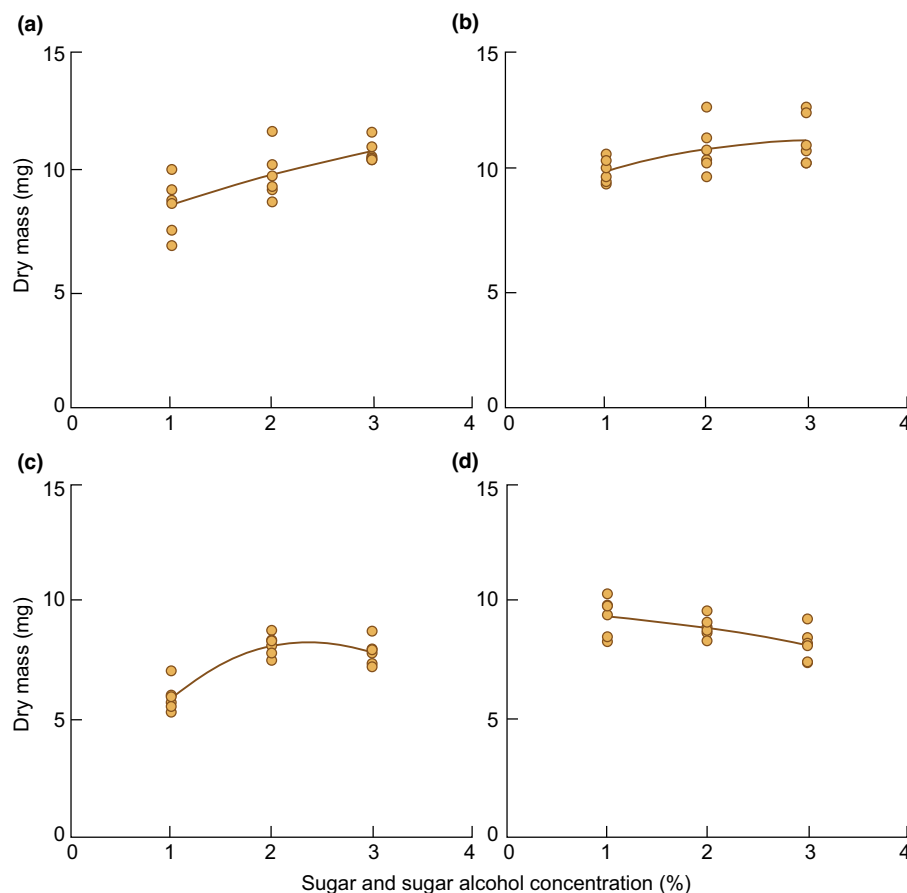


Fig. 5 Sugar and sugar alcohol-dependent cumulative growth of the mycobiont isolated from *Xanthoria parietina*. The response of fungal growth on solid Lilly-Barnett Medium (LBM) supplemented with either 1, 2 or 3% of (a) D-glucose ($R^2 = 0.55$), (b) D-mannitol ($R^2 = 0.31$), (c) D-arabitol ($R^2 = 0.80$) or (d) ribitol ($R^2 = 0.41$), as carbon source was assessed after 8 wk ($n = 6$ biological replicates); each dot represents a single value, dotted lines show trend curves. Data were re-analysed from fig. 5 in Pichler *et al.* (2021).

et al., 1989), and ribitol produced by the photobiont can be converted into arabitol and mannitol by *Ramalina* spp. mycobionts (Komiya & Shibata, 1971). Later, ribitol transport from the photobiont to the mycobiont, which can convert it into arabitol and return it back to the photobiont, was confirmed for *Ramalina yasudae* (Kosugi *et al.*, 2013). Mannitol and arabitol are present in mycobiont hyphae of many species, but glycerol, volemitol and erythritol only in a few (Honegger *et al.*, 1993). Generally, mycobionts apparently convert glucose into mannitol, and ribitol into arabitol and mannitol after import by the fungus, whereas erythritol may remain unchanged and moves only slowly between the symbionts (Richardson *et al.*, 1968; Komiya & Shibata, 1971; Spribille *et al.*, 2022).

Evidence suggests that D-arabitol improves the photobiont's ability to dissipate excess light energy during desiccation (Kosugi *et al.*, 2013), ribitol increase F_v/F_m , that is the photobiont's maximum quantum yield of photosystem II (Hájek *et al.*, 2009a), and both, mannitol and ribitol, enhance the solubilisation of fungal enzymes during freezing events, providing cryoprotection (Fontaniella *et al.*, 2000; Hájek *et al.*, 2009b). Therefore, the complex symbiotic interplay within a lichen thallus involves provision of carbon from the photobiont to the mycobiont, but also mutual stimulation of fitness parameters such as photosynthetic performance of the photobiont and enhanced freezing tolerance of the mycobiont.

Moreover, the release of sugars and sugar alcohols also benefits the lichen microbiota. Glucose, ribitol and mannitol can be taken up by Alphaproteobacteria associated with lichen photobionts (Kono *et al.*, 2017). Bacteria associated with the lichen *Leptogium puberulum* can catabolize degradation products of the lichen thallus, such as D-cellobiose and N-acetyl-D-glucosamine, mycobiont-specific D-mannitol and glucose-containing carbohydrates of the photobiont (Grzesiak *et al.*, 2021). Furthermore, lichenicolous fungi can metabolise mannitol and ribitol (Yoshino *et al.*, 2020). In return, components of the microbiota, for example basidiomycete yeasts, may contribute polysaccharides to the lichen matrix (Tagirdzhanova *et al.*, 2021). Therefore, sugars and sugar alcohols released by the primary lichen symbionts are likely attractants for other micro-organisms to join the symbiosis. Lichen sugars and sugar alcohols may also influence the composition of lichen-inhabiting bacteria, although chemical communication between the primary myco- and photobionts and lichen-inhabiting micro-organisms is not well-understood (Grimm *et al.*, 2021). In summary, sugars and sugar alcohols appear to be key molecules involved in the chemical crosstalk between lichen myco- and photobionts, from the precontact stage through to the fully established lichen thallus, with important roles for development, growth and fitness.

3. Phytohormones from the precontact stage through to the intact thallus

Phytohormones such as abscisic acid (ABA), auxins, brassinosteroids, cytokinins, ethylene, gibberellins, jasmonates, salicylic acid and strigolactones are pivotal signalling compounds in vascular plants, implicated in the regulation of plant growth, development

and stress response (Santner *et al.*, 2009). We use the widely accepted term 'phytohormone', but acknowledge that many phytohormones are derivatives of simple metabolic compounds and are not exclusive to vascular plants, but are also produced by bacteria, algae, fungi and bryophytes (Costacurta & Vanderleyden, 1995; Decker *et al.*, 2006; Tarakhovskaya *et al.*, 2007; Chanclud & Morel, 2016). Therefore, phytohormones must have evolved before the vascular plants. Unsurprisingly, they also exist in lichens. Phytohormones are involved in the molecular crosstalk between organisms across plants, fungi, micro-organisms and others, and hence, are inter- and multikingdom signalling compounds (Foo *et al.*, 2019), whose roles are much better understood in mycorrhiza – the symbiotic associations between plants and beneficial, mycorrhizal fungi (Zygomycota, Glomeromycota, Ascomycota, Basidiomycota) – than in lichens. In arbuscular mycorrhiza, involving penetration of the host's cell walls by the fungus, strigolactones exuded by plants roots attract fungi and influence fungal morphology and metabolism (Parniske, 2008). Arbuscular mycorrhiza can be promoted by ABA, auxins and strigolactones, suppressed by brassinosteroids, cytokinins and gibberellins (Pozo *et al.*, 2015; Liao *et al.*, 2018), and jasmonic acid and salicylic acid signalling can influence the expression of plant defence genes for plant pathogen resistance (Pozo & Azcón-Aguilar, 2007). Ectomycorrhiza between mycorrhizal fungi and perennial, usually woody plants, is also regulated by phytohormones (Basso *et al.*, 2020; Abdulsalam *et al.*, 2021). For example, the formation of the Hartig net between root cortex and rhizodermis cells can be stimulated by a key auxin, indole-3-acetic acid (IAA), which apparently represents one of the most abundant phytohormones involved in ectomycorrhizae (Ek *et al.*, 1983; Gay *et al.*, 1994). However, many questions remain open about the role of phytohormones in lichens.

As for other compounds involved in lichenization, a prerequisite for phytohormones to affect lichenization is that they are released into the extracellular space of the prospective symbionts. The sparse evidence available suggests that this is the case (see Table 2a for summary). For example, isolated myco- and photobionts of the lichens *Cetraria islandica* and *Cladonia rangiferina* can release ethylene and its precursor 1-aminocyclopropane-1-carboxylic acid (Schieleit & Ott, 1996). Indole-3-carbaldehyde, a precursor and degradation product of IAA (Bandurski *et al.*, 1995; Gazarian *et al.*, 1998), was present in the growth media of lichen-forming *Trebouxia* spp. and suppressed fungal spore germination and hyphal growth of the *Gyalolechia bracteata* mycobiont (Meeßen *et al.*, 2013). Indole-3-acetic acid was produced and extracellularly released by mycobionts and photobionts isolated from *Cladonia grayi*, *Tephromela atra* and *Xanthoria parietina* in a species- and (for photobionts) light-dependent manner (Table 2b), while production and release of ABA, gibberellin A3, indole-3-butyric acid and jasmonic acid depended on photobiont species, and of salicylic acid and jasmonic acid on mycobiont species (Pichler *et al.*, 2020a,b). Exogenously supplied IAA and indole-3-butyric acid stimulated growth of isolated mycobionts (Wang *et al.*, 2009, 2010) and exogenous IAA treatment increased the water contents of isolated lichen photobiont cultures, although more research is needed to evaluate whether the vacuole, the cytoplasm, the cell wall or the

Table 2 Evidence for extracellular phytohormone and cyclic peptide release by isolated lichen photo- and mycobionts.

Mycobiont Photobiont	Phytohormones	Cyclic peptides	References
(a)			
Cetraria islandica	ET	nd	Schieleit & Ott (1996)
<i>Coccomyxa</i> sp.	ET	nd	
Cladonia grayi	IAA, JA	nd	Pichler <i>et al.</i> (2020b)
<i>Asterochloris glomerata</i>	IAA, ABA, JA	nd	Pichler <i>et al.</i> (2020a)
Cladonia rangiferina	ET	nd	Schieleit & Ott (1996)
<i>Trebouxia irregularis</i>	ET	nd	
Fulgensia bracteata	nd	nd	Meeßen <i>et al.</i> (2013)
<i>Trebouxia</i> sp.	ICA	CTT	
Fulgensia fulgens	nd	nd	Meeßen <i>et al.</i> (2013)
<i>Trebouxia</i> sp.	ICA	CTT	
Lecidea lurida	nd	nd	Meeßen <i>et al.</i> (2013)
<i>Asterochloris</i> sp.	nd	CLT, CTT	
Tephromela atra	IAA, SA	nd	Pichler <i>et al.</i> (2020b)
<i>Trebouxia</i> sp.	IAA, ABA, JA, GA ₃ , IBA	nd	Pichler <i>et al.</i> (2020a)
Toninia sedifolia	nd	nd	Meeßen <i>et al.</i> (2013)
<i>Trebouxia</i> sp.	ICA	CTT	
Xanthoria elegans	nd	nd	Meeßen <i>et al.</i> (2013)
<i>Trebouxia</i> sp.	ICA	CTT	
Xanthoria parietina	IAA, SA	nd	Pichler <i>et al.</i> (2020b)
<i>Trebouxia decolorans</i>	IAA, ABA	nd	Pichler <i>et al.</i> (2020a)
(b)			
Mycobiont treatment	Cladonia grayi	Xanthoria parietina	Tephromela atra
Dim light	0.012 ± 0.012	1.158 ± 0.602	13.526 ± 2.580
Photobiont treatment	Asterochloris glomerata	Trebouxia decolorans	Trebouxia sp.
Dim light	0.973 ± 0.155	0.553 ± 0.051	1.290 ± 0.221
High light	1.317 ± 0.311	1.257 ± 0.205	1.280 ± 0.188
DH + dim light	2.927 ± 0.658	1.053 ± 0.407	6.531 ± 1.205
DH + high light	1.371 ± 0.111	0.942 ± 0.389	1.101 ± 0.269

(a) Phytohormones, phytohormone precursors and cyclic peptides released by isolated lichen symbionts. (b) Indole-3-acetic acid release rates by isolated lichen photo- and mycobionts. ABA, abscisic acid; CLT, cyclo-(L-leucyl-L-tyrosyl); CTT, cyclo-(L-tryptophyl-L-tryptophyl); ET, ethylene; GA₃, gibberellin A₃; IAA, indole-3-acetic acid; ICA, indole-carbaldehyde; IBA, indole-3-butyric acid; JA, jasmonic acid; nd, not determined. Bold and regular text in the left column indicates mycobiont and photobiont species, respectively. (b) Data show extracellular indole-3-acetic acid (IAA) release rates in nmol per day (d) by isolated lichen myco- and photobionts, normalised to dry mass (nmol IAA g⁻¹ d⁻¹). Values consider the degradation of IAA in the medium according to exponential degradation described by $y = 100 e^{-0.134x}$. Data were re-analysed from values in table 1 and fig. 4 of Pichler *et al.* (2020a), and figs 3 and 4 of Pichler *et al.* (2020b). Data are mean ± SD; DH, de-rehydration cycle.

intercellular water between the photobiont cells is responsible for the increased water contents (Pichler *et al.*, 2020b). Therefore, these few reports provide evidence that phytohormones released by prospective symbionts are present in the extracellular space, where they could be mutually perceived, supporting lichenization from the precontact stage onwards.

Studies on phytohormones in fully established lichen thalli, or released by them, provide evidence for the production of ABA, ethylene, gibberellin A₃, IAA and zeatin in lichens (Epstein *et al.*, 1986; Ott & Schieleit, 1994; Ergün *et al.*, 2002), and future studies will most likely show that this short list is rather incomplete. The physiological and metabolic response of lichens to exogenously offered phytohormones shows their relevance to the lichen symbiosis. Ethylene is thought to be involved in lichen response to abiotic stress factors, such as heavy metals (Garty *et al.*, 1997; Kauppi *et al.*, 1998), and is released by lichen thalli at elevated rates upon hydration (Ott & Schieleit, 1994). Thallus ABA production was enhanced upon rehydration of desiccated thalli (Dietz &

Hartung, 1998; Unal *et al.*, 2008) and exogenous ABA application improved thallus survival during permanent water saturation (Dietz & Hartung, 1999). In turn, exogenous ABA treatment can enhance the photobiont's desiccation tolerance, as shown for *Peltigera polydactylon* (Beckett *et al.*, 2005). In addition, proteins and genes involved in auxin biosynthesis of lichen-associated bacteria were identified, and bacterial auxins could also influence the growth of the main lichen symbionts (Grube *et al.*, 2009; Cernava *et al.*, 2017).

In summary, lichen myco- and photobionts, and components of the lichen microbiota, can produce various phytohormones, which can be released in significant amounts into the extracellular space, where they are available for perception by other micro-organisms, potentially affecting their gene expression, metabolism and physiology. The few papers available provide only limited insights, and care must be taken in the interpretation of the data. Future studies should comprise careful identification of the chemical structures of known, or perhaps new, phytohormones, by state-of-

the-art hyphenated techniques such as LC–MS/MS, and NMR, in combination with molecular approaches for testing hypotheses, such as using genetically modified myco- and photobionts, once sequenced, culturable and amenable to modification. Of the phytohormones studied so far, roles for ethylene in regulating stress response and for ABA in affecting fungal spore germination and hyphal growth seem to emerge. Moreover, ABA and IAA appear to influence lichen water relations, and thus, potentially desiccation tolerance.

IV. The thallus: a fine-tuned symbiotic interplay supporting nutrition, tolerance of irradiation and desiccation, and reproduction

1. Shunting of macro- and micronutrients in the lichen thallus

Whereas at least some knowledge is available regarding the shunting of phosphorous and nitrogen between lichen symbionts, the role of mineral nutrition in the various stages of lichenization still remains a black box that deserves illuminating through future studies. In resynthesized *Usnea hakonensis* thalli (Kono *et al.*, 2020), photobiont genes involved in photosynthate transport and mycobiont genes involved in nitrogen and phosphorus transport were upregulated compared with isolated myco- and photobionts. Specifically, gene clusters specific for acid phosphatase and phosphate transport were upregulated in the mycobiont, but absent in the photobiont, which upregulated genes for ATPase, arguing for transport of inorganic phosphates from the mycobiont to the photobiont. The upregulation of photosynthate transport reinforces that the photobiont is the main breadwinner providing reduced carbon to the symbiosis, and the mycobiont reciprocates by supplying the photobiont with other elements, such as phosphorous and nitrogen, calcium, magnesium, manganese, copper and iron extracted from the substrate through weathering (Adamo & Violante, 2000), and importantly, water (Honegger, 1991).

Tripartite lichens and cyanolichens benefit from nitrogen fixed by cyanobacteria (Honegger, 1993; Almendras *et al.*, 2018), involving significant symbiotic interaction. In a tripartite lichen, *Peltigera aphthosa*, nitrogen fixed by *Nostoc* sp. cyanobacteria was transferred to both, the mycobiont, which incorporated it into amino acids (e.g. alanine, aspartate, glutamine and glutamate), and the *Coccomyxa* photobiont. Over 95% was fixed as NH_4^+ , supported by cyanobacterial CH_3NH_3^+ and NH_4^+ -specific transporters and requiring the activity of glutamate dehydrogenase in cyanobacteria (Kershaw & Millbank, 1970; Rai *et al.*, 1980, 1983), although further evidence is needed for a better appreciation of the biochemical pathways involved in nitrogen fixation and transport, and their mutual regulation in the tripartite symbiosis. In addition, lichens also host potentially nitrogen-fixing eubacteria, whose diversity is greater in chlorolichens than in cyanolichens (Almendras *et al.*, 2018). Therefore, we suggest that in the differentiation stage, the microbiota could commence supporting the symbiosis, either by directly supplying nutrients to the primary symbionts or indirectly by nutrient release from a decaying microbiota fraction, that is from dead bacteria or fungi.

2. Secondary lichen products benefit the established symbiosis

Lichens can produce over 1000 known secondary metabolites, including polyketides such as anthraquinones, chromones, depsides, depsidones, lactones, usnic acid and xanthenes. As their occurrence and abundance often correlates well with phylogenetic lineages, they are used as a mainstay in lichen chemosystematics (Calcott *et al.*, 2018). Most polyketides are deposited as extracellular crystals on the hyphal cell walls in the upper cortex of the thallus, for example atranorin, parietin and usnic acid, or as granules within hyphae, for example melanins, whereas others accumulate in the medulla, for example physodic acid, physodalic acid, lecanoric acid and protocetraric acid (Solhaug & Gauslaa, 1996; Solhaug *et al.*, 2009; Suno *et al.*, 2021; Daminova *et al.*, 2022). However, it should be noted that not all fungi produce crystallized compounds in the lichenized thallus, notably cyanolichens. Polyketide biosynthetic pathways have not been fully characterized, but genes encoding polyketide synthases constitute one of the largest gene families in fungi, and recent genomic studies reflect the increasing interest in pathway genes generating these compounds (Singh *et al.*, 2021a,b; Gerasimova *et al.*, 2022). The antimicrobial, allelopathic and antiherbivore properties of many secondary lichen metabolites point to pivotal roles in protecting lichen thalli from biotic stress factors, including in fungus–fungus competition with common moulds, while those with other properties, such as antioxidant and photoprotective properties lend further support to the established symbiosis (Calcott *et al.*, 2018; for review). Protection from, and competition with other organisms contributes to shaping community structure and the distribution of individual lichen species (Armstrong & Welch, 2007). For example, lichen substances released into the environment can have allelopathic effects towards other fungi, bacteria, algae, mosses and vascular plants, and also other lichens via influencing photosynthesis, respiration, transpiration, protein and nucleic acid synthesis, membrane ion transport and permeability (Macias *et al.*, 2007). Lichen acids such as vulpinic acid, evernic acid and stictic acid exert allelopathic effects within lichen communities, although lichenized fungi have a higher tolerance as compared to non-lichenized fungi (Whiton & Lawrey, 1984). Usnic acid is toxic to non-lichen-forming microalgae such as *Chlamydomonas reinhardtii* (Schimmer & Lehner, 1973) and *Scenedesmus quadricauda* (Bačkor *et al.*, 2010), and the moss *Physcomitrella patens* (Goga *et al.*, 2020). Usnic acid, norstictic acid and parietin are also allelopathic to rock-inhabiting fungi, other green algae and cyanobacteria (Gazzano *et al.*, 2013). Lichen photobionts such as *Trebouxia erici* appear to tolerate usnic acid better, although exogenous application of usnic acid and vulpinic acid can inhibit photobiont growth (Bačkor *et al.*, 1998, 2010). Therefore, polyketides produced by lichen mycobionts help sustaining the symbiosis by protection from biotic stress factors, and may also play a role in regulating the number of photobiont cells within a thallus. On the contrary, certain polyketides such as atranorin, usnic acid, and anthraquinones produced by the mycobiont can act as ‘sun-screen’ pigments in the upper cortex, regulating the amount of incident light received by the photobiont (Solhaug *et al.*, 2003; Beckett *et al.*, 2021).

Polyketide production by axenically cultured mycobionts differs from that of resynthesizing myco- and photobiont co-cultures (Sveshnikova & Piercey-Normore, 2021) and does not necessarily correspond to the set of polyketides found in lichen thalli collected in nature. In addition, specific conditions of temperature, light and water availability, and growth media with added sugars or sugar alcohols appear to be required for the production of specific secondary metabolites (Stocker-Wörgötter, 2002; Elshobary *et al.*, 2016). Associated ascomycete or basidiomycete fungi can influence the production of secondary lichen metabolites (Spribille *et al.*, 2016; Asplund *et al.*, 2018), and in turn, secondary metabolites deposited extracellularly on a lichen can influence bacterial growth, and thus the composition of the lichen microbiota, as summarized by Grimm *et al.* (2021). The mere existence of the lichen microbiota implies that lichen-associated bacteria have adapted tolerance of lichen compounds. Interestingly, certain gene clusters encoding secondary metabolite synthases are downregulated in *Cladonia rangiferina* co-cultured with its compatible photobiont, suggesting that potential allelopathic effects towards the photobiont can be avoided in the early stages of lichenization (Sveshnikova & Piercey-Normore, 2021). Which compounds precisely are affected is not yet clear and considering the large number of gene clusters for biosynthetic pathways present in lichens genomes, linking all biosynthetic genes with products will be a tall order. The differential regulation of these genes is in accordance with the early finding of differential methylation patterns in secondary metabolite genes during lichenization (Armaleo & Miao, 1999). Taken together, the findings discussed in this section suggest that the production of the set of secondary metabolites typically found in a fully formed thallus requires stimuli from the environment, the photobiont and other co-habitants within the thallus. However, whereas secondary lichen metabolites are key to sustain the fully formed thallus, their downregulation in the early stages of lichenization implies that they are unwanted while establishing first contact. Whether the switch from down- to upregulating polyketide synthesis occurs already in the incorporation stage, the differentiation stage or even after establishing a co-habiting microbiota, is yet to be seen.

3. Molecules conferring desiccation tolerance

A key trait of fully established lichen thalli is their remarkable ability to survive desiccation. Desiccation tolerance is often defined as the capability of surviving drying to the air-dry state at relative humidities below 65%, corresponding to a drop in absolute water content to or below $0.1 \text{ g H}_2\text{O g}^{-1}$ dry mass and a water potential of $\leq -100 \text{ MPa}$, and is believed to occur in almost all, if not all, lichens (Kranner *et al.*, 2008). Such extremely low water contents can induce 'vitrification', that is transition to a 'glassy state', of cytoplasmic components dominated by proteins and sugars, causing metabolism to come to a standstill (Kranner *et al.*, 2008; Candotto Carniel *et al.*, 2021). This state of 'suspended life' supports survival under extreme environmental conditions, such as circumpolar habitats that are often dominated by lichens – which is why lichens are often called 'extremophiles', as they can thrive under environmental conditions too harsh for vascular plants that

rapidly outcompete the slow-growing lichens under more favourable conditions.

Desiccation is associated with oxidative damage, molecular crowding and Maillard reactions causing damage to macromolecules and subcellular organization, and surviving with almost no water is based on constitutive and inducible protection (Kranner *et al.*, 2008; Candotto Carniel *et al.*, 2016; Oliver *et al.*, 2020). Briefly, known protection mechanisms include an efficient antioxidant system, 'late embryogenesis abundant' (LEA)-like proteins or 'dehydrins' and 'desiccation related proteins' (DRPs), nonreducing sugars and sugar alcohols (Kranner *et al.*, 2005, 2008, 2010; Banchi *et al.*, 2018). Together with other 'natural deep eutectic solvents' (NADES), these compounds may form 'functional liquid media' capable of dissolving molecules of low water solubility (du Toit *et al.*, 2021), supporting survival during and after drying. LEA-like proteins likely act as molecular shields and together with non-reducing sugars, substitute for water, maintaining the spacing between and within macromolecules (Tunnacliffe & Wise, 2007), avoiding molecular crowding, Maillard reactions and cellular collapse upon desiccation. While a 'dual role' for lichen sugar alcohols, being main carbon sources and contributing to desiccation tolerance, has recently been reviewed (Kranner *et al.*, 2022; Spribille *et al.*, 2022), the involvement of protective proteins in lichen desiccation tolerance has not been investigated, although postulated earlier (Kranner & Lutzoni, 1999), and supported by the finding that constitutively expressed LEA-like proteins are present in *Trebouxia* photobionts (Candotto Carniel *et al.*, 2016). Furthermore, hydrophobins, small cysteine-rich, hydrophobic proteins on the cell surface of the mycobiont, could play important roles in keeping air-filled spaces within stratified foliose and fruticose lichen thalli during water saturation, potentially contributing to oxidative stress avoidance (Honegger, 1993).

Extracellularly deposited lichen polyketides (polyphenols especially) have significant *in vitro* antioxidant activities (Fernández-Moriano *et al.*, 2016). However, survival of desiccation requires scavenging of reactive oxygen species formed upon desiccation by intracellular antioxidants, especially in the light. It is not known whether antioxidants are released extracellularly by free-living lichen mycobionts and photobionts, but evidence exists that lichenization is accompanied by mutual upregulation of protective systems, shown for the lichen *Cladonia vulcani* (Kranner *et al.*, 2005). Without fungal contact, the alga *Trebouxia excentrica* tolerates only very dim light and its carotenoid-based photoprotection is only partially effective, and without the alga, the glutathione-based antioxidant system of the fungus is slow and ineffective. However, in the fully formed thallus, antioxidant and photoprotective mechanisms are more effective by orders of magnitude compared with its isolated partners (Kranner *et al.*, 2005), vaguely reminiscent of a very different symbiosis, in which photosynthetic sea slugs induce protective changes (such as elevating the plastoquinone pool) to the chloroplasts they steal from algae (Havurinne & Tyystjärvi, 2020). This mutually enhanced resistance to oxidative stress together with enhanced desiccation tolerance supports life above ground in the form of the *Cladonia vulcani* miniature skyscraper, increasing the chance of dispersal of

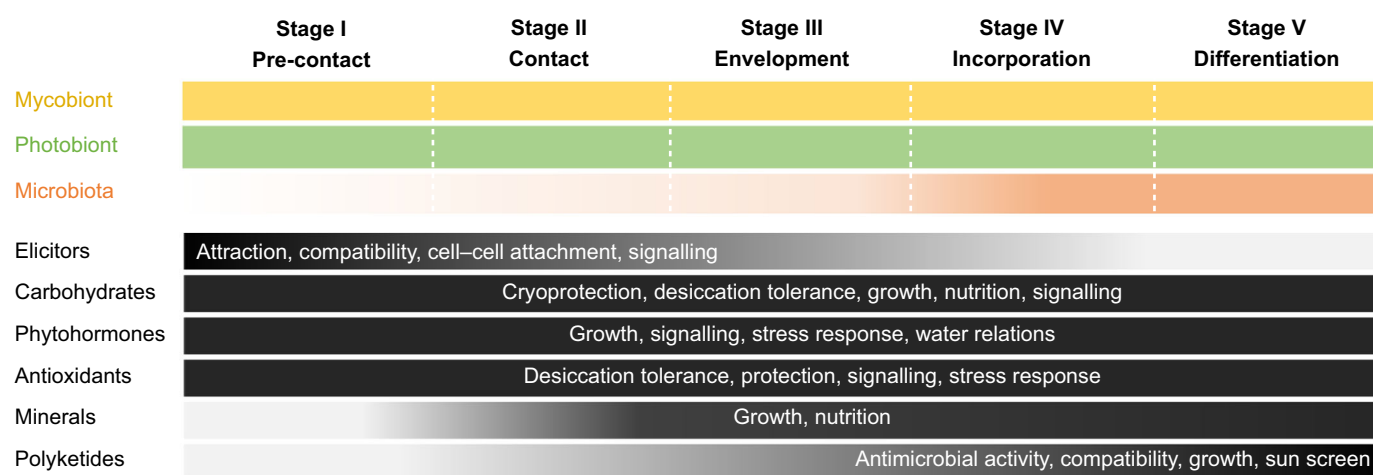


Fig. 6 Summary of metabolite groups and signalling molecules proposed to be involved in lichenization. Compounds involved in the five stages of lichenization, none of which are envisaged to be exclusive to a specific phase, are represented in shades from grey to black; darker shades denote a proposed greater contribution to lichenization, also in the bar representing the microbiota. Compounds that elicit contact, such as lectins and cyclic peptides, appear to dominate the early stages, whereas carbon exchange between the symbionts, phytohormone signalling and protection from oxidative damage are suggested to be involved in the lichenization process throughout all stages, whereas polyketides and nutrition with non-carbon-based elements such as nitrogen and phosphorous support establishment and maintenance of the thallus, together with the microbiota acquired during and after completion of lichenization.

reproductive propagules and ensuring the symbionts' joint evolutionary success (Kranner *et al.*, 2005). The ability of prospective lichen symbionts to survive desiccation already before lichenization is a very likely pre-requisite for them to form a lichen. However, the symbiosis appears to prime the symbionts for becoming even harder towards the environmental changes, such as elevated irradiance and air dryness, associated with leaving their hidden life below ground, or in shaded habitats such as soil, bark or rock. Moreover, desiccation tolerance requires great cell wall flexibility to support recurrent cellular shrinkage and swelling upon desiccation and rehydration, as shown for isolated lichen photobionts (Gonzalez-Hourcade *et al.*, 2020). Whereas information on the responses of lichens and, less so, on their isolated primary symbionts, to desiccation and rehydration is available, the effects on the lichen microbiota are still to be investigated.

V. Outlook

Many symbioses are formed by including a symbiotic partner in an already established host, or a specific organ of that host, for example roots, symbiosomes and other parts of a macrosymbiont. Lichens differ from these cases, as their thalli arise as a symbiotic structure from the very beginning of a microbial encounter, which requires recognition of the partners and maintenance of the partnership from the earliest stages onwards. Here, we summarized the current knowledge about key metabolites apparently involved in this process. The emerging picture suggests that various metabolite groups work in a concerted manner throughout the various stages of lichenization, with elicitors including lectins and certain cyclic peptides – and surely many other molecules will be found to contribute – supporting contact in the early stages of lichenization. Sugars and sugar alcohols, being the main carbon sources first involved in attracting a prospective mycobiont and later shunted

between the symbionts, are very likely pivotal players throughout all stages of lichenization, together with other compound groups, such as secondary lichen products and antioxidants, that contribute to maintaining the fine-tuned symbiotic interplay within the fully established thallus, and in participation with the lichen microbiota (Fig. 6). This suggests a shifting spectrum of regulatory metabolites from the first encounter of the partners through to stabilizing the fully established lichen thallus. It will be interesting to study, whether and in which way the above-mentioned metabolite groups contribute to epigenetic regulation of gene expression through DNA methylation, as such a regulation has already been demonstrated in other symbioses (e.g. Satge *et al.*, 2016; Li *et al.*, 2018). As lichens often grow under austere conditions, it would seem plausible to assume a sustained epigenetic adjustment of symbiotic transcriptomes, a sort of mutual parameter setting, and this may comprise a mutual down-regulation of defence systems to enable contact. Furthermore, thalli evolved in distantly related fungi, and there are different trajectories to what has convergently evolved in more complex stratified forms. Certainly, much more work lies ahead of us to better understand the process of lichenization from the precontact stage to basal or complex thalli, and the role of metabolites in lichenization. We are convinced that a plethora of further metabolites are yet to be identified, given the tremendous diversity of lichens and the fact that they involve rather different lineages of fungi.

Acknowledgements

We would like to thank Thomas Roach, Erwann Arc and two anonymous referees for their valuable comments, and the Austrian Science Fund (FWF), project P32092-B32 granted to IK, and the Italien-Zentrum of the University of Innsbruck to GP for financial support.

Competing interests

None declared.

ORCID

Fabio Candotto Carniel  <https://orcid.org/0000-0002-8277-4725>

Martin Grube  <https://orcid.org/0000-0001-6940-5282>

Ilse Kranner  <https://orcid.org/0000-0003-4959-9109>

Lucia Muggia  <https://orcid.org/0000-0003-0390-6169>

Gregor Pichler  <https://orcid.org/0000-0002-3129-7834>

Data availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

References

- Abdulsalam O, Wagner K, Wirth S, Kunert M, David A, Kallenbach M, Boland W, Kothe E, Krause K. 2021. Phytohormones and volatile organic compounds, like geosmin, in the ectomycorrhiza of *Tricholoma vaccinum* and Norway spruce (*Picea abies*). *Mycorrhiza* 31: 173–188.
- Adamo P, Violante P. 2000. Weathering of rocks and neogenesis of minerals associated with lichen activity. *Applied Clay Science* 16: 229–256.
- Ahmadjian V, Jacobs JB. 1981. Relationship between fungus and alga in the lichen *Cladonia cristatella* Tuck. *Nature* 289: 169–172.
- Alen'kina SA, Bogatyrev VA, Matora LY, Sokolova MK, Chernyshova MP, Trutneva KA, Nikitina VE. 2014. Signal effects of the lectin from the associative nitrogen-fixing bacterium *Azospirillum brasilense* Sp7 in bacterial-plant root interactions. *Plant and Soil* 381: 337–349.
- Almendras K, García J, Carú M, Orlando J. 2018. Nitrogen-fixing bacteria associated with *Peltigera* cyanolichens and *Cladonia* chlorolichens. *Molecules* 23: 3077.
- Armaleo D, Miao V. 1999. Symbiosis and DNA methylation in the *Cladonia* lichen fungus. *Symbiosis* 26: 143–163.
- Armaleo D, Müller O, Lutzoni F, Andrésson ÓS, Blanc G, Bode HB, Collart FR, Dal Grande F, Dietrich F, Grigoriev IV *et al.* 2019. The lichen symbiosis reviewed through the genomes of *Cladonia grayi* and its algal partner *Asterochloris glomerata*. *BMC Genomics* 20: 605.
- Armstrong RA, Welch AR. 2007. Competition in lichen communities. *Symbiosis* 43: 1–12.
- Asplund J, Gauslaa Y, Merinero S. 2018. Low synthesis of secondary compounds in the lichen *Lobaria pulmonaria* infected by the lichenicolous fungus *Plectocarpon lichenum*. *New Phytologist* 217: 1397–1400.
- Athukorala SNP, Huebner E, Piercey-Normore MD. 2014. Identification and comparison of the 3 early stages of resynthesis for the lichen *Cladonia rangiferina*. *Canadian Journal of Microbiology* 60: 41–52.
- Bačkor M, Hudak J, Repčák M, Ziegler W, Bačkorová M. 1998. The influence of pH and lichen metabolites (vulpinic acid and (+) usnic acid) on the growth of the lichen photobiont *Trebouxia irregularis*. *The Lichenologist* 30: 577–582.
- Bačkor M, Klemová K, Bačkorová M, Ivanova V. 2010. Comparison of the phytotoxic effects of usnic acid on cultures of free-living alga *Scenedesmus quadricauda* and aposymbiotically grown lichen photobiont *Trebouxia erici*. *Journal of Chemical Ecology* 36: 405–411.
- Banchi E, Candotto Carniel F, Montagner A, Petruzzellis F, Pichler G, Giarola V, Bartels D, Pallavicini A, Tretiach M. 2018. Relation between water status and desiccation-affected genes in the lichen photobiont *Trebouxia gelatinosa*. *Plant Physiology and Biochemistry* 129: 189–197.
- Bandurski RS, Cohen JD, Slovin JP, Reinecke DM. 1995. Auxin biosynthesis and metabolism. In: Davies PJ, ed. *Plant hormones: physiology, biochemistry and molecular biology*. Dordrecht, the Netherlands: Springer, 39–65.
- Basso V, Kohler A, Miyauchi S, Singan V, Guinet F, Šimura J, Novák O, Barry KW, Amirebrahimi M, Block J *et al.* 2020. An ectomycorrhizal fungus alters sensitivity to jasmonate, salicylate, gibberellin, and ethylene in host roots. *Plant, Cell & Environment* 43: 1047–1068.
- Becker B. 2013. Snow ball earth and the split of Streptophyta and Chlorophyta. *Trends in Plant Science* 18: 180–183.
- Beckett RP, Mayaba N, Minibayeva FV, Alyabyev AJ. 2005. Hardening by partial dehydration and ABA increase desiccation tolerance in the cyanobacterial lichen *Peltigera polydactylon*. *Annals of Botany* 96: 109–115.
- Beckett RP, Minibayeva F, Solhaug KA, Roach T. 2021. Photoprotection in lichens: adaptations of photobionts to high light. *The Lichenologist* 53: 21–33.
- Bornet E. 1872. Sur les gonidies des lichens. *Comptes Rendus* 74: 820.
- Brunauer G, Hager A, Grube M, Türk R, Stocker-Worgotter E. 2007. Alterations in secondary metabolism of aposymbiotically grown mycobionts of *Xanthoria elegans* and cultured resynthesis stages. *Plant Physiology and Biochemistry* 45: 146–151.
- Bubrick P, Galun M. 1980. Proteins from the lichen *Xanthoria parietina* which bind to phycobiont cell walls. Correlation between binding patterns and cell wall cytochemistry. *Protoplasma* 104: 167–173.
- Bubrick P, Galun M, Frensdorff A. 1984. Observations on free-living *Trebouxia* de Puymaly and *Pseudotreboxia* Archibald, and evidence that both symbionts from *Xanthoria parietina* (L.) TH. FR. can be found free-living in nature. *New Phytologist* 97: 455–462.
- Calcott MJ, Ackerley DF, Knight A, Keyzers RA, Owen JG. 2018. Secondary metabolism in the lichen symbiosis. *Chemical Society Reviews* 47: 1730–1760.
- Candotto Carniel F, Fernandez-Marín B, Arc E, Craighero T, Laza JM, Incerti G, Tretiach M, Kranner I. 2021. How dry is dry? Molecular mobility in relation to thallus water content in a lichen. *Journal of Experimental Botany* 72: 1576–1588.
- Candotto Carniel F, Gerdol M, Montagner A, Banchi E, De Moro G, Manfrin C, Muggia L, Pallavicini A, Tretiach M. 2016. New features of desiccation tolerance in the lichen photobiont *Trebouxia gelatinosa* are revealed by a transcriptomic approach. *Plant Molecular Biology* 91: 319–339.
- Cernava T, Erlacher A, Aschenbrenner IA, Krug L, Lassek C, Riedel K, Grube M, Berg G. 2017. Deciphering functional diversification within the lichen microbiota by meta-omics. *Microbiome* 5: 82.
- Chanclud E, Morel JB. 2016. Plant hormones: a fungal point of view. *Molecular Plant Pathology* 17: 1289–1297.
- Costacurta A, Vanderleyden J. 1995. Synthesis of phytohormones by plant-associated bacteria. *Critical Reviews in Microbiology* 21: 1–18.
- Daminova AG, Rogov AM, Rassabina AE, Beckett RP, Minibayeva FV. 2022. Effect of melanization on thallus microstructure in the lichen *Lobaria pulmonaria*. *Journal of Fungi* 8: 791.
- De Los RA, Ascaso C, Grube M. 2002. An ultrastructural, anatomical and molecular study of the lichenicolous lichen *Rimularia insularis*. *Mycological Research* 106: 946–953.
- Decker EL, Frank W, Sarnighausen E, Reski R. 2006. Moss systems biology en route: phytohormones in *Physcomitrella* development. *Plant Biology* 8: 397–405.
- Delaux PM, Radhakrishnan GV, Jayaraman D, Cheem J, Malbreil M, Volkening JD, Sekimoto H, Nishiyama T, Melkonian M, Pokorny L *et al.* 2015. Algal ancestor of land plants was preadapted for symbiosis. *Proceedings of the National Academy of Sciences, USA* 112: 13390–13395.
- Díaz EM, Sánchez-Elordi E, Santiago R, Vicente C, Legaz ME. 2016. Algal-fungal mutualism: cell recognition and maintenance of the symbiotic status of lichens. *Journal of Veterinary Medicine and Research* 3: 1052.
- Díaz EM, Vicente-Manzanares M, Sacristán M, Vicente C, Legaz ME. 2011. Fungal lectin of *Peltigera canina* induces chemotropism of compatible *Nostoc* cells by constriction-relaxation pulses of cyanobiont cytoskeleton. *Plant Signaling & Behavior* 6: 1525–1536.
- Dietz S, Hartung W. 1998. Abscissic acid in lichens: variation, water relations and metabolism. *New Phytologist* 138: 99–106.
- Dietz S, Hartung W. 1999. The effect of abscissic acid on chlorophyll fluorescence in lichens under extreme water regimes. *New Phytologist* 143: 495–501.
- Douglas AE. 2014. *The symbiotic habit*. Princeton, NJ, USA: Princeton University Press.
- Drew EA, Smith DC. 1967. Studies in the physiology of lichens. VII. The physiology of the *Nostoc* symbiont of *Peltigera polydactyla* compared with cultured and free-living forms. *New Phytologist* 66: 379–388.

- Eisenreich W, Knispel N, Beck A. 2011. Advanced methods for the study of the chemistry and the metabolism of lichens. *Phytochemistry Reviews* 10: 445–456.
- Ek M, Ljungquist PO, Stenström E. 1983. Indole-3-acetic acid production by mycorrhizal fungi determined by gas chromatography-mass spectrometry. *New Phytologist* 94: 401–407.
- Elshobary ME, Osman ME, Abo-Shady AM, Komatsu E, Perreault H, Sorensen J, Piercey-Normore MD. 2016. Algal carbohydrates affect polyketide synthesis of the lichen-forming fungus *Cladonia rangiferina*. *Mycologia* 108: 646–656.
- Epstein E, Sagee O, Cohen JD, Garty J. 1986. Endogenous auxin and ethylene in the lichen *Ramalina duriae*. *Plant Physiology* 82: 1122–1125.
- Ergün N, Topcuoglu SF, Yildiz A. 2002. Auxin (indole-3-acetic acid), gibberellic acid (GA₃), abscisic acid (ABA) and cytokinin (zeatin) production by some species of mosses and lichens. *Turkish Journal of Botany* 26: 13–18.
- Erlacher A, Cernava T, Cardinale M, Soh J, Sensen CW, Grube M, Berg G. 2015. Rhizobiales as functional and endosymbiotic members in the lichen symbiosis of *Lobaria pulmonaria* L. *Frontiers in Microbiology* 6: 53.
- Fernández-Moriano C, Gómez-Serranillos MP, Crespo A. 2016. Antioxidant potential of lichen species and their secondary metabolites. A systematic review. *Pharmaceutical Biology* 54: 1–17.
- Fontaniella B, Vicente C, Legaz ME. 2000. The cryoprotective role of polyols in lichens: effects on the redistribution of RNase in *Evernia prunastri* thallus during freezing. *Plant Physiology and Biochemistry* 38: 621–627.
- Foo E, Plett JM, Lopez-Raez JA, Reid D. 2019. Editorial: the role of plant hormones in plant-microbe symbioses. *Frontiers in Plant Science* 10: 1391.
- Galun M, Braun A, Frensdorff A, Galun E. 1976. Hyphal walls of isolated lichen fungi - Autoradiographic localization of precursor incorporation and binding of fluorescein-conjugated lectins. *Archives of Microbiology* 108: 9–16.
- Garty J, Kloog N, Wolfson R, Cohen Y, Karnieli A, Avni A. 1997. The influence of air pollution on the concentration of mineral elements, on the spectral reflectance response and on the production of stress-ethylene in the lichen *Ramalina duriae*. *New Phytologist* 137: 587–597.
- Gay G, Normand L, Marmeisse R, Sotta B, Debaud JC. 1994. Auxin overproducer mutants of *Hebeloma cylindrosporum* Romagnesi have increased mycorrhizal activity. *New Phytologist* 128: 645–657.
- Gazarian IG, Lagrimini LM, Mellon FA, Naldrett MJ, Ashby GA, Thorneley RNF. 1998. Identification of skatolyl hydroperoxide and its role in the peroxidase-catalysed oxidation of indol-3-yl acetic acid. *Biochemical Journal* 333: 223–232.
- Gazzano C, Favero-Longo SE, Iacomussi P, Piervittori R. 2013. Biocidal effect of lichen secondary metabolites against rock-dwelling microcolonial fungi, cyanobacteria and green algae. *International Biodeterioration & Biodegradation* 84: 300–306.
- Gerasimova JV, Beck A, Werth S, Resl P. 2022. High diversity of type I polyketide genes in *Bacidia rubella* as revealed by the comparative analysis of 23 lichen genomes. *Journal of Fungi* 8: 449.
- Goga M, Elečko J, Marcincinová M, Ručová D, Bačkorová M, Bačkor M. 2020. Lichen metabolites: an overview of some secondary metabolites and their biological potential. In: Mérillon J-M, Ramawat KG, eds. *Co-evolution of secondary metabolites*. Cham, Switzerland: Springer International Publishing, 175–209.
- Gonzalez-Hourcade M, Braga MR, del Campo EM, Ascaso C, Patino C, Casano LM. 2020. Ultrastructural and biochemical analyses reveal cell wall remodelling in lichen-forming microalgae submitted to cyclic desiccation-rehydration. *Annals of Botany* 125: 459–469.
- Grimm M, Grube M, Schiefelbein U, Zühlke D, Bernhardt J, Riedel K. 2021. The lichens' microbiota, still a mystery? *Frontiers in Microbiology* 12: 623839.
- Grube M, Berg G. 2009. Microbial consortia of bacteria and fungi with focus on the lichen symbiosis. *Fungal Biology Reviews* 23: 72–85.
- Grube M, Cardinale M, de Castro JV, Müller H, Berg G. 2009. Species-specific structural and functional diversity of bacterial communities in lichen symbioses. *ISME Journal* 3: 1105–1115.
- Grzesiak J, Woltyńska A, Zdanowski MK, Górniak D, Świątecki A, Olech MA, Aleksandrak-Piekarczyk T. 2021. Metabolic fingerprinting of the Antarctic cyanolichen *Leptogium puberulum*-associated bacterial community (Western Shore of Admiralty Bay, King George Island, Maritime Antarctica). *Microbial Ecology* 82: 818–829.
- Hájek J, Váci P, Barták M. 2009a. Photosynthetic electron transport at low temperatures in the green algal foliose lichens *Lasallia pustulata* and *Umbilicaria hirsuta* affected by manipulated levels of ribitol. *Photosynthetica* 47: 199–205.
- Hájek J, Váci P, Barták M, Smejkal L, Lipavská H. 2009b. Cryoprotective role of ribitol in *Xanthoparmelia somloensis*. *Biologia Plantarum* 53: 677–684.
- Hametner C, Stocker-Wörgötter E, Grube M. 2014. New insights into diversity and selectivity of trentepohlialean lichen photobionts from the extratropics. *Symbiosis* 63: 31–40.
- Havurinne V, Tyystjärvi E. 2020. Photosynthetic sea slugs induce protective changes to the light reactions of the chloroplasts they steal from algae. *eLife* 9: 29.
- Hawksworth DL, Grube M. 2020. Lichens redefined as complex ecosystems. *New Phytologist* 227: 1281–1283.
- Hill DJ. 1989. The control of the cell cycle in microbial symbionts. *New Phytologist* 112: 175–184.
- Hill DJ. 1993. The co-ordination of development of symbionts in mutualistic symbiosis with reference to the cell cycle of the photobiont in lichens. *Symbiosis* 14: 325–333.
- Hill DJ, Smith DC. 1972. Lichen physiology. XII. 'The inhibition technique'. *New Phytologist* 71: 15–30.
- Honegger R. 1986. Ultrastructural studies in lichens. I. Haustorial types and their frequencies in a range of lichens with trebouxoid photobionts. *New Phytologist* 103: 785–795.
- Honegger R. 1991. Functional aspects of the lichen symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology* 42: 553–578.
- Honegger R. 1993. Developmental biology of lichens. *New Phytologist* 125: 659–677.
- Honegger R. 2012. 15 The symbiotic phenotype of lichen-forming ascomycetes and their endo- and epibionts. In: Hock B, ed. *Fungal associations*. Berlin, Heidelberg, Germany: Springer, 287–339.
- Honegger R, Edwards D, Axe L. 2013. The earliest records of internally stratified cyanobacterial and algal lichens from the lower devonian of the Welsh borderland. *New Phytologist* 197: 264–275.
- Honegger R, Kutasi V, Ruffner HP. 1993. Polyol patterns in eleven species of aposymbiotically cultured lichen mycobionts. *Mycological Research* 97: 35–39.
- Insarova ID, Blagoveshchenskaya EY. 2016. Lichen symbiosis: search and recognition of partners. *Biology Bulletin* 43: 408–418.
- Joneson S, Armaleo D, Lutzoni F. 2011. Fungal and algal gene expression in early developmental stages of lichen-symbiosis. *Mycologia* 103: 291–306.
- Joneson S, Lutzoni F. 2009. Compatibility and thigmotropism in the lichen symbiosis: a reappraisal. *Symbiosis* 47: 109–115.
- Kauppi M, Kauppi A, Garty J. 1998. Ethylene produced by the lichen *Cladonia stellaris* exposed to sulphur and heavy-metal-containing solutions under acidic conditions. *New Phytologist* 139: 537–547.
- Kershaw KA, Millbank JW. 1970. Nitrogen metabolism in lichens II. The partition of cephalodial fixed nitrogen between the mycobiont and phycobionts of *Peltigera aphthosa*. *New Phytologist* 69: 75–79.
- Komiyama T, Shibata S. 1971. Polyols produced by the cultured phyco- and mycobionts of some *Ramalina* species. *Phytochemistry* 10: 695–699.
- Kono M, Kon Y, Ohmura Y, Satta Y, Terai Y. 2020. In vitro resynthesis of cyanolization reveals the genetic background of symbiosis-specific fungal-algal interaction in *Usnea hakonensis*. *BMC Genomics* 21: 671.
- Kono M, Tanabe H, Ohmura Y, Satta Y, Terai Y. 2017. Physical contact and carbon transfer between a lichen-forming *Trebouxia* alga and a novel Alphaproteobacterium. *Microbiology* 163: 678–691.
- Kosugi M, Miyake H, Yamakawa H, Shibata Y, Miyazawa A, Sugimura T, Satoh K, Itoh S, Kashino Y. 2013. Arabinol provided by lichenous fungi enhances ability to dissipate excess light energy in a symbiotic green alga under desiccation. *Plant and Cell Physiology* 54: 1316–1325.
- Kranner I, Beckett R, Hochman A, Nash TH. 2008. Desiccation-tolerance in lichens: a review. *Bryologist* 111: 576–593.
- Kranner I, Cram WJ, Zorn M, Wornik S, Yoshimura I, Stabenheiner E, Pfeifhofer HW. 2005. Antioxidants and photoprotection in a lichen as compared with its isolated symbiotic partners. *Proceedings of the National Academy of Sciences, USA* 102: 3141–3146.
- Kranner I, Lutzoni F. 1999. Evolutionary consequences of transition to a lichen symbiotic state and physiological adaptation to oxidative damage associated with poikilohydry. In: Lerner HR, ed. *Plant responses to environmental stresses*:

- from phytohormones to genome reorganization. New York, NY, USA: Routledge, 38.
- Kranner I, Minibayeva FV, Beckett RP, Seal CE. 2010. What is stress? Concepts, definitions and applications in seed science. *New Phytologist* 188: 655–673.
- Kranner I, Pichler G, Grube M. 2022. The lichen market place. *New Phytologist* 234: 1541–1543.
- Lawrey JD, Diederich P. 2003. Lichenicolous fungi: interactions, evolution, and biodiversity. *Bryologist* 106: 80–120.
- Legaz ME, Fontaniella B, Millanes AM, Vicente C. 2004. Secreted arginases from phylogenetically far-related lichen species act as cross-recognition factors for two different algal cells. *European Journal of Cell Biology* 83: 435–446.
- Li Y, Liew YJ, Cui G, Czieielski MJ, Zahran N, Michell CT, Voolstra CR, Aranda M. 2018. DNA methylation regulates transcriptional homeostasis of algal endosymbiosis in the coral model *Aiptasia*. *Science Advances* 4: eaat2142.
- Liao DH, Wang SS, Cui MM, Liu JH, Chen AQ, Xu GH. 2018. Phytohormones regulate the development of arbuscular mycorrhizal symbiosis. *International Journal of Molecular Sciences* 19: 3146.
- Lines CEM, Ratcliffe RG, Rees TAV, Southon TE. 1989. A ^{13}C NMR study of photosynthate transport and metabolism in the lichen *Xanthoria calcicola* Oxner. *New Phytologist* 111: 447–456.
- Lücking R, Hodgkinson BP, Leavitt SD. 2016. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota: approaching one thousand genera. *Bryologist* 119: 361–416.
- Lutzoni F, Pagel M, Reeb V. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* 411: 937–940.
- Macias FA, Molinillo JMG, Varela RM, Galindo JCG. 2007. Allelopathy – a natural alternative for weed control. *Pest Management Science* 63: 327–348.
- Meeßen J, Eppenstein S, Ott S. 2013. Recognition mechanisms during the pre-contact state of lichens: II. Influence of algal exudates and ribitol on the response of the mycobiont of *Fulgensia bracteata*. *Symbiosis* 59: 131–143.
- Meeßen J, Ott S. 2013. Recognition mechanisms during the pre-contact state of lichens: I. Mycobiont-photobiont interactions of the mycobiont of *Fulgensia bracteata*. *Symbiosis* 59: 121–130.
- Molina MC, Vicente C. 1995. Correlations between enzymatic activity of lectins, putrescine content and chloroplast damage in *Xanthoria parietina* phycobionts. *Cell Adhesion and Communication* 3: 1–12.
- Muggia L, Baloch E, Stabenheimer E, Grube M, Wedin M. 2011. Photobiont association and genetic diversity of the optionally lichenized fungus *Schizoxylon albescentis*. *FEMS Microbiology Ecology* 75: 255–272.
- Muggia L, Fleischhacker A, Kopun T, Grube M. 2016. Extremotolerant fungi from alpine rock lichens and their phylogenetic relationships. *Fungal Diversity* 76: 119–142.
- Nelsen MP, Lücking R, Boyce CK, Lumbsch HT, Ree RH. 2020. No support for the emergence of lichens prior to the evolution of vascular plants. *Geobiology* 18: 3–13.
- Oliver MJ, Farrant JM, Hilhorst HWM, Mundree S, Williams B, Bewley JD. 2020. Desiccation tolerance: avoiding cellular damage during drying and rehydration. In: Merchant SS, ed. *Annual Review of Plant Biology*, vol. 71. Palo Alto, CA, USA: Annual Reviews, 435–460.
- Ott S, Schieleit P. 1994. Influence of exogenous factors on ethylene production by lichens. I. Influence of water content and water status conditions on ethylene production. *Symbiosis* 16: 187–201.
- Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* 6: 763–775.
- Peksa O, Geboušková T, Škvorová Z, Vančurová L, Škaloud P. 2022. The guilds in green algal lichens—an insight into the life of terrestrial symbiotic communities. *FEMS Microbiology Ecology* 98: 1–17.
- Pichler G, Carniel FC, Muggia L, Holzinger A, Tretiach M, Kranner I. 2021. Enhanced culturing techniques for the mycobiont isolated from the lichen *Xanthoria parietina*. *Mycological Progress* 20: 797–808.
- Pichler G, Stöggel W, Candotto Carniel F, Muggia L, Ametrano CG, Holzinger A, Tretiach M, Kranner I. 2020a. Abundance and extracellular release of phytohormones in aero-terrestrial microalgae (Trebouxiophyceae, Chlorophyta) as a potential chemical signaling source. *Journal of Phycology* 56: 1295–1307.
- Pichler G, Stöggel W, Trippel D, Candotto Carniel F, Muggia L, Ametrano CG, Cimen T, Holzinger A, Tretiach M, Kranner I. 2020b. Phytohormone release by three isolated lichen mycobionts and the effects of indole-3-acetic acid on their compatible photobionts. *Symbiosis* 82: 95–108.
- Pozo MJ, Azcón-Aguilar C. 2007. Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* 10: 393–398.
- Pozo MJ, López-Ráez JA, Azcón-Aguilar C, García-Garrido JM. 2015. Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. *New Phytologist* 205: 1431–1436.
- Rai AN, Rowell P, Stewart WDP. 1980. NH_4^+ assimilation and nitrogenase regulation in the lichen *Peltigera aphthosa* Willd. *New Phytologist* 85: 545–555.
- Rai AN, Rowell P, Stewart WDP. 1983. Interactions between cyanobacterium and fungus during $^{15}\text{N}_2$ -incorporation and metabolism in the lichen *Peltigera canina*. *Archives of Microbiology* 134: 136–142.
- Resl P, Bujold AR, Tagirdzhanova G, Meidl P, Rallo SF, Kono M, Fernández-Brime S, Guðmundsson H, Andr sson OS, Muggia L *et al.* 2022. Large differences in carbohydrate degradation and transport potential among lichen fungal symbionts. *Nature Communications* 13: 2634.
- Richardson DH, Hill DJ, Smith DC. 1968. Lichen physiology: XI. The role of the alga in determining the pattern of carbohydrate movement between lichen symbionts. *New Phytologist* 67: 469–486.
- Romero JAF, Paglini MG, Priano C, Koroch A, Rodr guez Y, Sailer J, Teleshova N. 2021. Algal and cyanobacterial lectins and their antimicrobial properties. *Marine Drugs* 19: 687.
- Sacrist n M, Millanes AM, Legaz ME, Vicente C. 2006. A lichen lectin specifically binds to the α -1,4-polygalactoside moiety of urease located in the cell wall of homologous algae. *Plant Signaling & Behavior* 1: 23–27.
- Sanders WB. 2002. In situ development of the foliicolous lichen *Phyllophiala* (Trichotheliaceae) from propagule germination to propagule production. *American Journal of Botany* 89: 1741–1746.
- Sanders WB, L cking R. 2002. Reproductive strategies, relichenization and thallus development observed *in situ* in leaf-dwelling lichen communities. *New Phytologist* 155: 425–435.
- Santner A, Calderon-Villalobos LIA, Estelle M. 2009. Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology* 5: 301–307.
- Satge C, Moreau S, Sallet E, Lefort G, Auriac MC, Remblier C, Cottret L, Gallardo K, Noirot C, Jardinaud MF *et al.* 2016. Reprogramming of DNA methylation is critical for nodule development in *Medicago truncatula*. *Nature Plants* 2: 16166.
- Schieleit P, Ott S. 1996. Ethylene production and 1-aminocyclopropane-1-carboxylic acid content of lichen bionts. *Symbiosis* 21: 223–231.
- Schimmer O, Lehner H. 1973. Untersuchungen zur Wirkung von Usnins ure auf die Gr nalge *Chlamydomonas reinhardtii*. *Archiv f r Mikrobiologie* 93: 145–154.
- Schwendener S. 1869. *Die Algentypen der Flechtengonidien: Programm f r Rectoratsfeier der Universit t*. Basel, Switzerland: Universit tsbuchdruckerei von C. Schultze.
- Selosse MA, Le Tacon F. 1998. The land flora: a phototroph–fungus partnership? *Trends in Ecology & Evolution* 13: 15–20.
- Selosse MA, Strullu-Derrien C, Martin FM, Kamoun S, Kenrick P. 2015. Plants, fungi and oomycetes: a 400-million year affair that shapes the biosphere. *New Phytologist* 206: 501–506.
- Singh G, Armaleo D, Dal Grande F, Schmitt I. 2021a. Depside and depsidone synthesis in lichenized fungi comes into focus through a genome-wide comparison of the olivetoric acid and physodic acid chemotypes of *Pseudevernia furfuracea*. *Biomolecules* 11: 1445.
- Singh G, Calchera A, Schulz M, Drechsler M, Bode HB, Schmitt I, Dal Grande F. 2021b. Climate-specific biosynthetic gene clusters in populations of a lichen-forming fungus. *Environmental Microbiology* 23: 4260–4275.
- Singh RS, Walia AK. 2014. Characteristics of lichen lectins and their role in symbiosis. *Symbiosis* 62: 123–134.
- Solhaug KA, Gauslaa Y. 1996. Parietin, a photoprotective secondary product of the lichen *Xanthoria parietina*. *Oecologia* 108: 412–418.
- Solhaug KA, Gauslaa Y, Nybakken L, Bilger W. 2003. UV-induction of sun-screening pigments in lichens. *New Phytologist* 158: 91–100.
- Solhaug KA, Lind M, Nybakken L, Gauslaa Y. 2009. Possible functional roles of cortical depsides and medullary depsidones in the foliose lichen *Hypogymnia*

- physodes. Flora – Morphology, Distribution, Functional Ecology of Plants* 204: 40–48.
- Spribille T. 2018. Relative symbiont input and the lichen symbiotic outcome. *Current Opinion in Plant Biology* 44: 57–63.
- Spribille T, Resl P, Stanton DE, Tagirdzhanova G. 2022. Evolutionary biology of lichen symbioses. *New Phytologist* 234: 1566–1582.
- Spribille T, Tuovinen V, Resl P, Vanderpool D, Wolinski H, Aime MC, Schneider K, Stabenheiner E, Toome-Heller M, Thor G *et al.* 2016. Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353: 488–492.
- Stocker-Wörgötter E. 2001a. Experimental studies of the lichen symbiosis: DNA-analyses, differentiation and secondary chemistry of selected mycobionts, artificial resynthesis of two- and tripartite symbioses. *Symbiosis* 30: 207–227.
- Stocker-Wörgötter E. 2001b. New frontiers in bryology and lichenology: experimental lichenology and microbiology of lichens: culture experiments, secondary chemistry of cultured mycobionts, resynthesis, and thallus morphogenesis. *Bryologist* 104: 576–581.
- Stocker-Wörgötter E. 2002. Investigating the production of secondary compounds in cultured lichen mycobionts. In: Kranner IC, Beckett RP, Varma AK, eds. *Protocols in lichenology: culturing, biochemistry, ecophysiology and use in biomonitoring*. Berlin, Heidelberg, Germany: Springer, 296–306.
- Stocker-Wörgötter E, Türk R. 1991. Artificial resynthesis of thalli of the cyanobacterial lichen *Peltigera praetextata* under laboratory conditions. *The Lichenologist* 23: 127–138.
- Suno H, Machida M, Dohi T, Ohmura Y. 2021. Quantum chemical calculation studies toward microscopic understanding of retention mechanism of Cs radioisotopes and other alkali metals in lichens. *Scientific Reports* 11: 8228.
- Sveshnikova N, Piercey-Normore MD. 2021. Transcriptome comparison of secondary metabolite biosynthesis genes expressed in cultured and lichenized conditions of *Cladonia rangiferina*. *Diversity-Basel* 13: 529.
- Tagirdzhanova G, Saary P, Tingley JP, Díaz-Escandón D, Abbott DW, Finn RD, Spribille T. 2021. Predicted input of uncultured fungal symbionts to a lichen symbiosis from metagenome-assembled genomes. *Genome Biology and Evolution* 13: 18.
- Tarakhovskaya ER, Maslov YI, Shishova MF. 2007. Phytohormones in algae. *Russian Journal of Plant Physiology* 54: 163–170.
- du Toit SF, Bentley J, Farrant JM. 2021. NADES formation in vegetative desiccation tolerance: prospects and challenges. In: Verpoorte R, Witkamp GJ, Choi YH, eds. *Eutectic solvents and stress in plants*. London, UK: Academic Press–Elsevier Science, 225–252.
- Trembley ML, Ringli C, Honegger R. 2002. Morphological and molecular analysis of early stages in the resynthesis of the lichen *Baeomyces rufus*. *Mycological Research* 106: 768–776.
- Tschermak-Woess E. 1978. *Myrmecia reticulata* as a phycobiont and free-living – free-living *Trebouxia* – the problem of *Stenocybe septata*. *The Lichenologist* 10: 69–79.
- Tunnacliffe A, Wise MJ. 2007. The continuing conundrum of the LEA proteins. *Naturwissenschaften* 94: 791–812.
- Unal D, Senkardesler A, Sukatar A. 2008. Absciscic acid and polyamine contents in the lichens *Pseudevernia furfuracea* and *Ramalina farinacea*. *Russian Journal of Plant Physiology* 55: 115–118.
- Vivas M, Sacristán M, Legaz ME, Vicente C. 2010. The cell recognition model in chlorolichens involving a fungal lectin binding to an algal ligand can be extended to cyanolichens. *Plant Biology* 12: 615–621.
- Wang XY, Wei XL, Luo H, Kim JA, Jeon HS, Koh YJ, Hur JS. 2010. Plant hormones promote growth in lichen-forming fungi. *Mycobiology* 38: 176–179.
- Wang Y, Han KS, Wang XY, Koh YJ, Hur JS. 2009. Effect of ribitol and plant hormones on aposymbiotical growth of the lichen-forming fungi of *Ramalina farinacea* and *Ramalina fastigiata*. *Mycobiology* 37: 28–30.
- Wang YY, Liu B, Zhang XY, Zhou QM, Zhang T, Li H, Yu YF, Zhang XL, Hao XY, Wang M *et al.* 2014. Genome characteristics reveal the impact of lichenization on lichen-forming fungus *Endocarpon pusillum* Hedwig (Verrucariales, Ascomycota). *BMC Genomics* 15: 34.
- Whiton JC, Lawrey JD. 1984. Inhibition of crustose lichen spore germination by lichen acids. *Bryologist* 87: 42–43.
- Yoshimura I, Kurokawa T, Yamamoto Y, Kinoshita Y. 1993. Development of lichen thalli *in vitro*. *Bryologist* 96: 412–421.
- Yoshino K, Yamamoto K, Hara K, Sonoda M, Yamamoto Y, Sakamoto K. 2019. The conservation of polyol transporter proteins and their involvement in lichenized Ascomycota. *Fungal Biology* 123: 318–329.
- Yoshino K, Yamamoto K, Masumoto H, Degawa Y, Yoshikawa H, Harada H, Sakamoto K. 2020. Polyol-assimilation capacities of lichen-inhabiting fungi. *The Lichenologist* 52: 49–59.