

# CHAPTER 19-1

## BACTERIAL EFFECTS ON BRYOPHYTES

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# CHAPTER 19-1

## BACTERIAL EFFECTS ON BRYOPHYTES



Figure 1. Nodules of the nitrogen-fixing bacterium *Bradyrhizobium* with mosses on *Acacia koa*. Photo courtesy of James Leary.

This is the most exciting chapter I have written thus far! The study of bacterial interactions between bryophytes and bacteria is quite new, and fascinating relationships are unfolding.

Nomenclature for phyla in this are from Oren and Garrity (2021) (see Euzéby 1997)

There have been few explorations of the bacteria that are naturally associated with bryophytes (Koua *et al.* 2015). Koua and coworkers explored the bacteria on eight bryophyte species. They identified 42 bacterial species in 90 DGGE gel bands. The bacterial genus *Clostridium* (Figure 2) predominated, comprising 21.4% of the total bacterial community.

Bacteria could influence their bryophyte substrates in a number of ways. For dead and dying bryophytes, they could contribute to decomposition. For living bryophytes, they could block light needed for photosynthesis. But at the same time they could produce CO<sub>2</sub> through respiration, contributing to higher photosynthetic rates. But beyond these more easily conceived roles, they can contribute hormones and other substances that might influence the development of the bryophytes or the community where

they both live. And even more interesting relationships are unfolding.

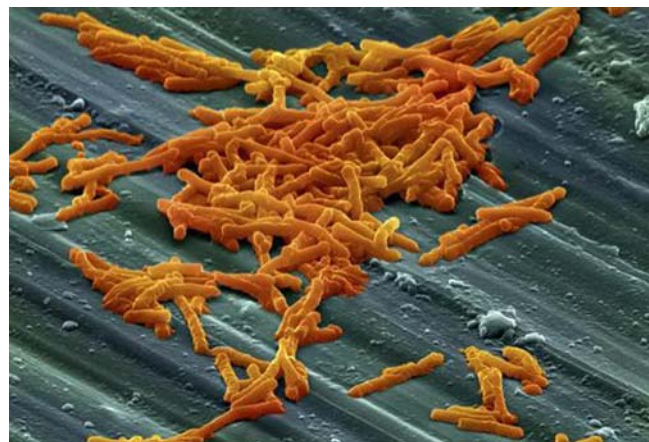


Figure 2. *Clostridium difficile*, a predominant bacterial genus on some bryophytes. Photo through Creative Commons.



## Bacteria Communities on Bryophytes

During and van Tooren (1990, 2008) reminded the ecologists that bryophytes in the ecosystem may be influenced by their interactions with other organisms, including bacteria. Such interactions might involve mineral nutrition, carbon economy, herbivory, and growth and development of gametophytes.

Among the abundant bacteria associated with bryophytes in Japan are strains of *Burkholderia* (ubiquitous obligately aerobic, rod-shaped, Gram-negative, genus of **Pseudomonadota** (previously Proteobacteria); Figure 3), *Hafnia* (facultatively anaerobic, rod-shaped, Gram-negative genus of **Pseudomonadota**; Figure 4), *Methanobacterium* (nonmotile, anaerobic genus of **Archaea**; Figure 5), *Methylobacterium* (pink-pigmented, facultatively anaerobic, straight rod-shaped, Gram-negative genus of **Pseudomonadota**; Figure 6), *Pantoea* (yellow-pigmented, Gram-negative genus of **Pseudomonadota**; Figure 7), and *Serratia* (facultatively anaerobic, rod-shaped, Gram-negative genus of **Pseudomonadota**; Figure 8), occurring as endophytes, epiphytes, or both (Opelt & Berg 2004; Bragina *et al.* 2013; Koua *et al.* 2015).



Figure 3. *Burkholderia pseudomallei*; *Burkholderia* is one of the abundant bryophyte-dwelling bacterial genera in Japan. Photo by Gavin Koh, through Creative Commons.

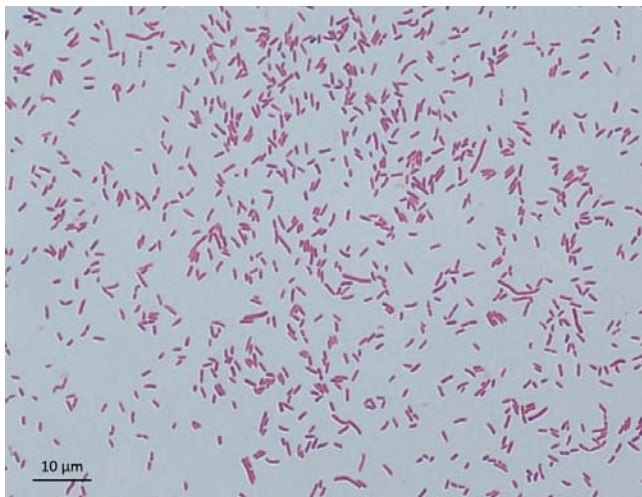


Figure 4. *Hafnia alvei*, in one of the abundant bryophyte-dwelling bacterial genera in Japan. Photo by Antoine2003, through Creative Commons.

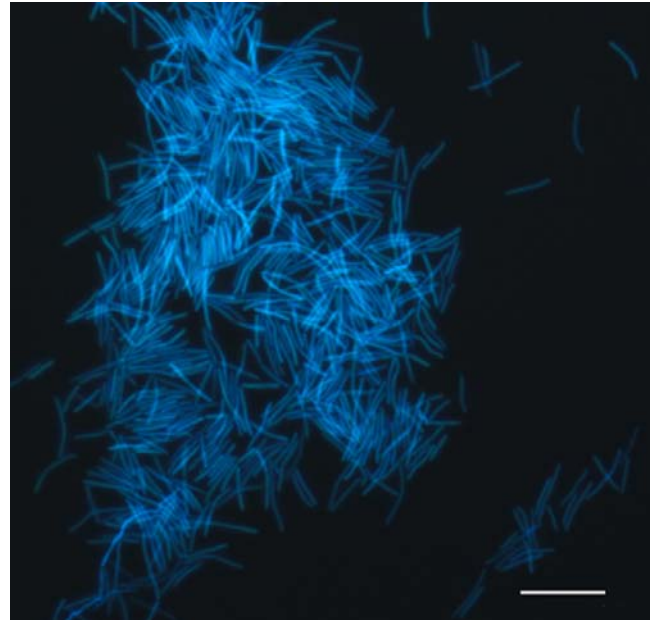


Figure 5. *Methanobacterium* sp., one of the abundant bryophyte-dwelling bacterial genera in Japan. Photo from JAMSTEC, through Creative Commons.

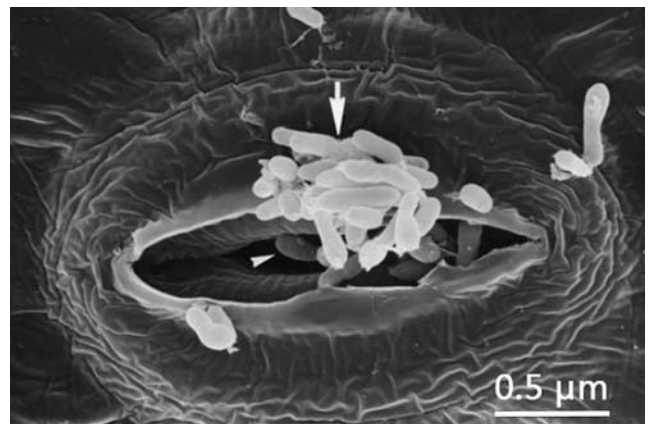


Figure 6. *Methylobacterium* sp. in sunflower stoma, one of the abundant bryophyte-dwelling bacterial genera in Japan. Photo by U. Kutschera, through Wikimedia Commons.

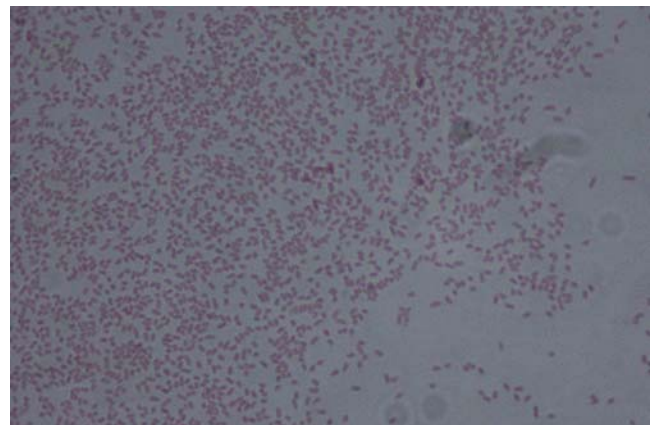


Figure 7. *Pantoea agglomerans* Gram stain, a species that occurs on bryophytes and is antagonistic toward some pathogenic bacteria and fungi. Photo by Dr. Sahay, through Creative Commons.





Figure 8. *Serratia marcescens* bacteria on bread slice; *Serratia* is an abundant genus on bryophytes in Japan and is antagonistic toward them. Photo by DBN, through Creative Commons.

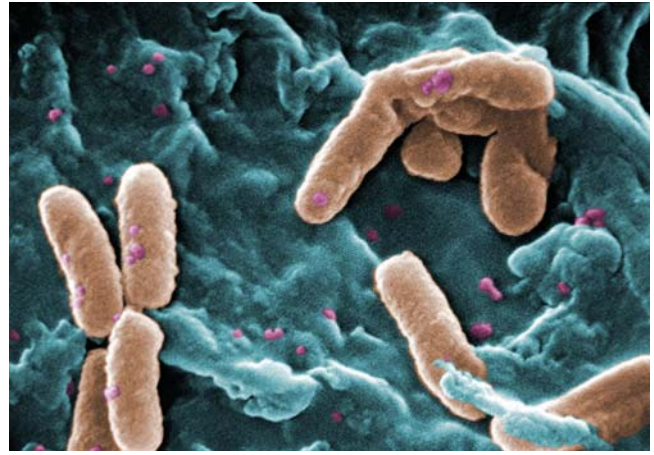


Figure 10. *Pseudomonas aeruginosa*; *Pseudomonas putida* is antagonistic toward bryophytes. Photo by Janice Haney Carr, CDC, through Public domain.

On the other hand, some bacteria are antagonistic toward the bryophytes, including species such as *Bacillus* sp. (*Bacillota* – syn. = *Firmicutes*; Figure 9), *Pseudomonas putida* (*Pseudomonadota*; see Figure 10), *Serratia* sp. (Figure 8), and *Xanthomonas* sp. (*Pseudomonadota*; Figure 11) (Opelt *et al.* 2007). *Serratia liquefaciens* (see Figure 8), predominant in the mosses *Sphagnum* (Figure 12) and *Aulacomnium* (Figure 13), and *Serratia proteamaculans* (see Figure 8) are the most effective antagonists among the bacterial isolates from these same mosses (Opelt & Berg 2004).



Figure 11. *Xanthomonas oryzae* pv. *oryzicola* infecting a leaf; some members of this bacterial genus are antagonistic toward bryophytes.. Photo by S. Q. An *et al.*, through Creative Commons.

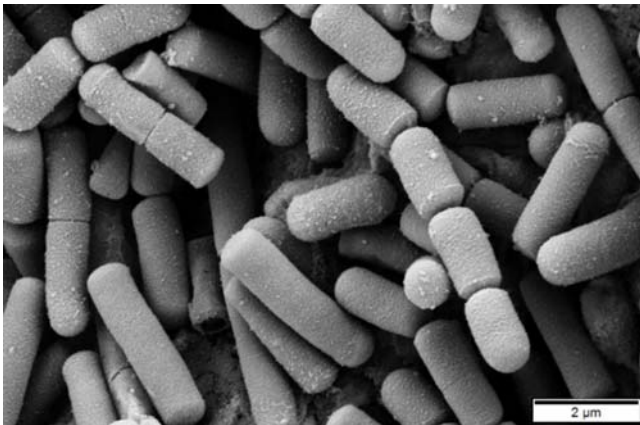


Figure 9. *Bacillus cereus* SEM, in an abundant genus on bryophytes in Japan and antagonistic toward them. Photo by Mogana Das Murtey and Patchamuthu Ramasamy, through Creative Commons.



Figure 12. *Sphagnum* blanket bog, habitat for *Serratia liquefaciens*, one of the strongest antagonists against bryophytes. Photo through Creative Commons.





Figure 13. *Aulacomnium palustre*, habitat for *Serratia liquefaciens*, one of the strongest antagonists against bryophytes. Photo by Hermann Schachner, through Creative Commons.

Koua and coworkers (2015) found bacterial colonizers of bare-rock bryophytes in their Japanese collections to be  $\gamma$ -Proteobacteria (Pseudomonadota) [*Buttiauxella*, *Enterobacter* (Figure 14), *Erwinia* (Figure 15), *Pantoea* (Figure 7), *Pseudomonas* (Figure 10), and *Salmonella* (Figure 16)] and Bacillota [*Anaerobacter* (Figure 17), *Clostridium* (Figure 2)] – a group that can survive extreme conditions, especially desiccation, through production of endospores. *Citrobacter* (Pseudomonadota; Figure 18), *Clostridium* (Bacillota), *Pseudomonas* (Pseudomonadota), and *Serratia* (Figure 8) were common among highly populated soil and bare-rock-associated bryophytes. *Anaerobacter* (Bacillota), *Buttiauxella* (Pseudomonadota), *Erwinia*, and *Pantoea* were limited to the bryophytes associated with bare rocks.

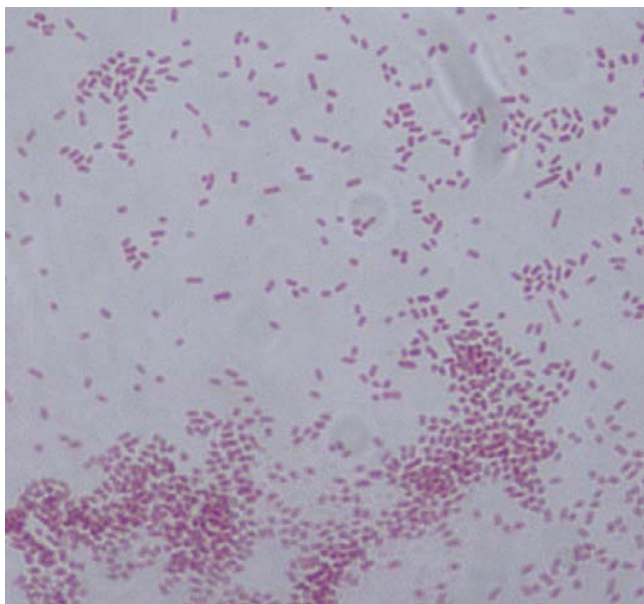


Figure 14. *Enterobacter aerogenes*; the genus *Enterobacter* is a bacterial colonizer of bare-rock bryophytes in Japan. Photo by Riraq25, through Creative Commons.



Figure 15. *Erwinia tracheiphila* causing flower wilt; the genus *Erwinia* is a bacterial colonizer of bare-rock bryophytes in Japan. Photo by Howard F. Schwartz, through Creative Commons.

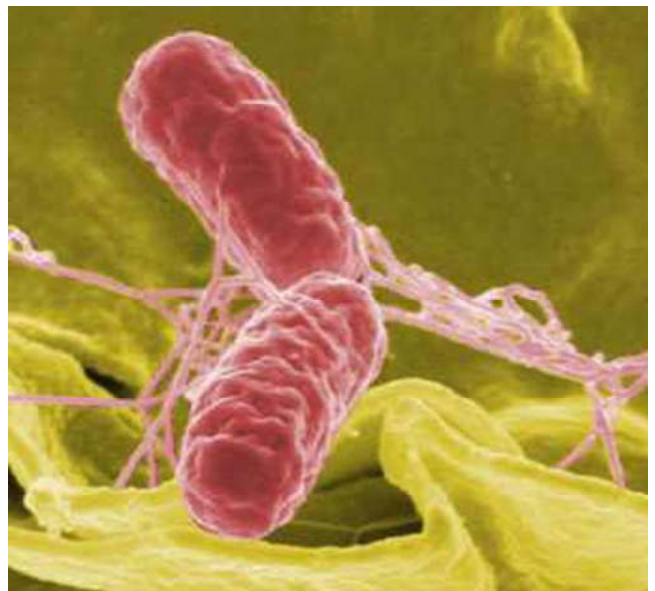


Figure 16. *Salmonella*, bacterial colonizer of bare-rock bryophytes in Japan. Photo by JohnnyMrNinja, through Creative Commons.

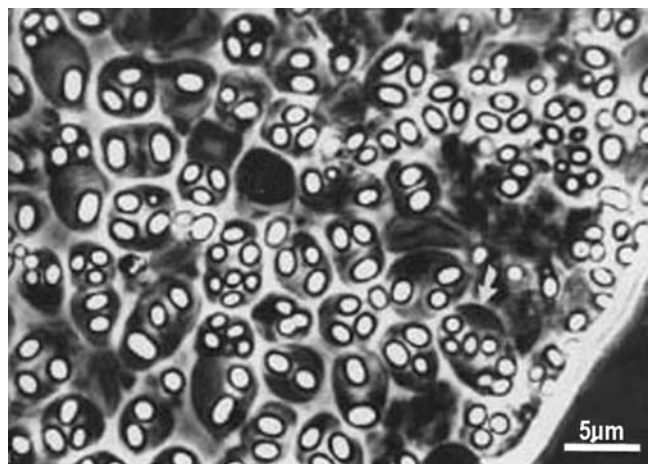


Figure 17. *Anaerobacter polyendosporus*; members of this genus can survive extreme conditions, especially desiccation, through production of endospores. Photo by Abtop, through Creative Commons.



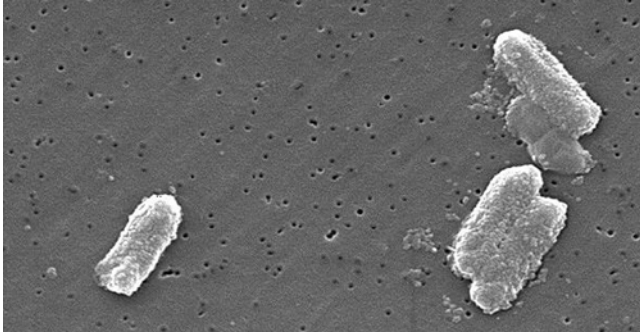


Figure 18. *Citrobacter freundii* SEM; some members of *Citrobacter* are common bacteria among highly populated soil and bare-rock-associated bryophytes. Photo through public domain.

Some bryophyte-dwelling bacteria, especially **Proteobacteriaceae**, are fussy, selecting only bryophytes of highly populated soil habitats: *Dickeya* (Figure 19), *Klebsiella* (Figure 20), *Obesumbacterium*, and *Pectobacterium* (Figure 21) (Koua *et al.* 2015). *Serratia proteamaculans* (see Figure 8) occurred exclusively in the moss *Trachycystis microphylla* (Figure 22) of both bare rocks and highly populated soils. These contrast with *Clostridium* (**Bacillota**; Figure 2), which was present on all species of bryophytes in all habitats in the Japanese study.



Figure 19. *Dickeya cf. dadantii* or *Pectobacterium carotovorum* on onion; some species of *Dickeya* are selective for bryophytes of highly populated soil plots. Photo through Creative Commons.

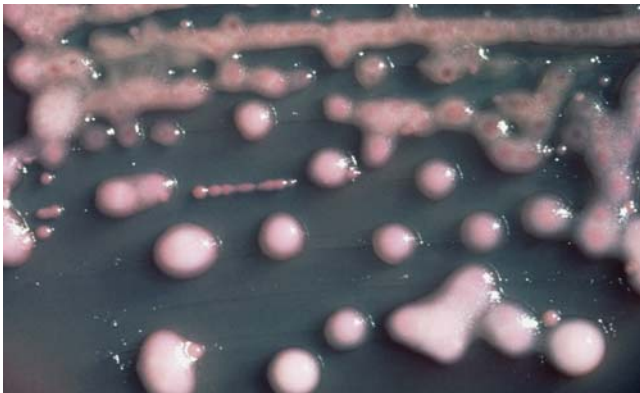


Figure 20. *Klebsiella pneumoniae* pink colonies; some species of *Klebsiella* are selective for bryophytes of highly populated soil plots. Photo from CDC, through public domain.



Figure 21. *Pectobacterium carotovorum* on lettuce; some species of *Pectobacterium* are selective for bryophytes of highly populated soil plots. Photo Gerald Holmes, Strawberry Center, Cal Poly San Luis Obispo, through Creative Commons.



Figure 22. *Trachycystis microphylla*; *Serratia proteamaculans* occurred exclusively on this moss species in a Japanese study. Photo by Harum Koh, through Creative Commons.

Scheirer and Dolan (1983) found an unidentified bacterium, similar to *Agrobacterium* (**Pseudomonadota**; Figure 23), on both surfaces of *Polytrichum commune* (Figure 24) leaves. The terminal cells of the moss lamellae act like a pseudoepidermis (Figure 25), providing a microhabitat suitable for the bacteria and other microorganisms. The bacteria did not occur in the cell interiors.

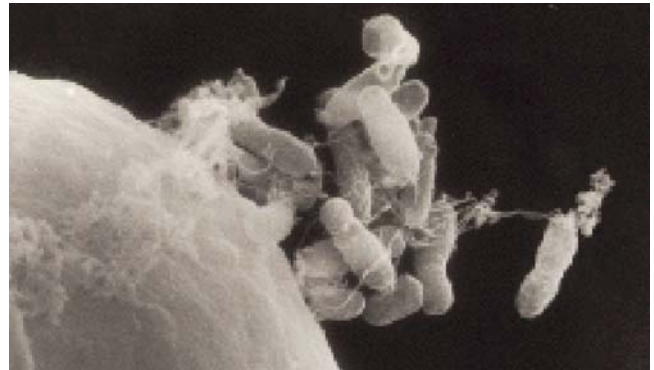


Figure 23. *Agrobacterium tumefaciens*; an unidentified bacterium similar to *Agrobacterium*, occurs on both surfaces of *Polytrichum commune* leaves. Photo through Creative Commons.





Figure 24. *Polytrichum commune*; an unidentified bacterium similar to *Agrobacterium* occurs on both surfaces of leaves of this moss. Photo by Bob Klips, with permission.

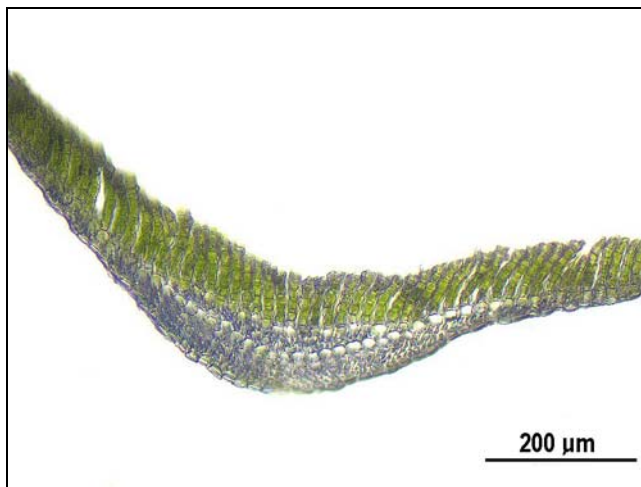


Figure 25. *Polytrichum commune* leaf section showing lamellae that act like an epidermis. Photo by Kristian Peters, through Creative Commons.

Tang *et al.* (2016) again noted that our understanding of the relationships of the abundant bacteria on bryophyte hosts is largely lacking. They analyzed the bacterial community associated with ten liverwort and ten moss host species in Tibet, China. They found no obvious differences in bacterial richness between mosses and liverworts. Nevertheless, the diversity was significantly higher with liverworts than with mosses. The bacteria that were most constantly present were members of the phyla **Acidobacteriota**, **Actinomycetota**, **Armatimonadota**, **Bacteroidota**, **Planctomycetota**, and **Pseudomonadota**. Those in the phyla **Chloroflexota**, **Fibrobacterota**, **Gemmatimonadota**, and **Chlamydiota** appeared among only some of the bryophytes. The most constant genera among the bryophytes were *Burkholderia* (**Pseudomonadota**; Figure 3), *Frankia* (**Actinomycetota**; Figure 26), *Froniathitans*, *Granulicella* (**Acidobacteriota**), *Hafnia* (Figure 4), *Haliangium* (**Pseudomonadota**; Figure 27), *Mucilaginibacter* (**Bacteroidota**), *Novosphingobium* (**Pseudomonadota**; Figure 28), *Rhizobacter* (**Pseudomonadota**), and *Sorangium* (**Pseudomonadota**). Eleven of the bacteria couldn't be classified, suggesting that there may be many new bacteria to be identified among the bryophytes. Tang

and coworkers concluded that the phylogeny of hosts has a strong influence on the associated bacterial community and that niche also plays an important role when the hosts are phylogenetically more similar.



Figure 26. *Frankia alni* nodules on *Alnus glutinosa* roots; members of *Frankia* are among the most common genera on bryophytes in Tibet. Photo by Cwmhiraeth, through Creative Commons.

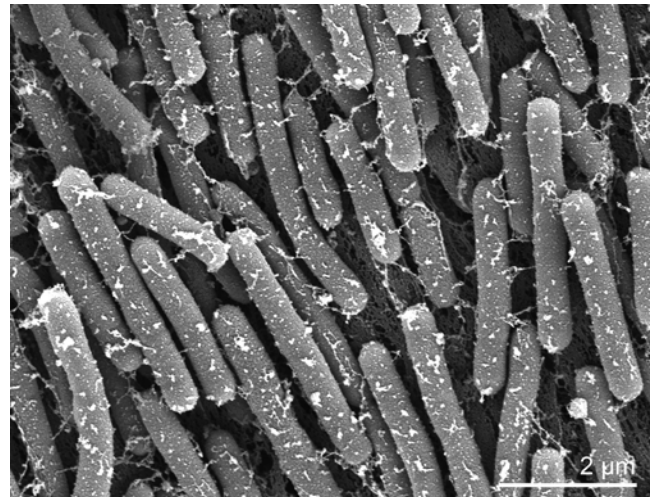


Figure 27. *Haliangium ochraceum*, in one of the most common genera of bacteria among bryophytes in Tibet. Photo by Manfred Rohde, through Creative Commons.

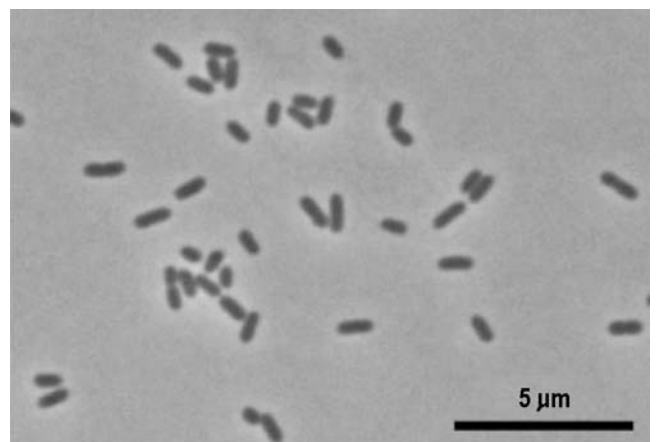


Figure 28. *Novosphingobium*, one of the most constant genera among bryophytes in Tibet. Photo by Nierychlo *et al.*, through Creative Commons.



Schauer and Kutschera (2013) concluded that some methylobacteria (Figure 6) prefer to colonize bryophytes. *Methylobacterium funariae* (see Figure 6) was described as a new species from *Funaria hygrometrica* (Figure 29). Further evidence suggests that *Methylobacterium* species (Figure 6) prefer gametophytes (1n tissues), including liverwort and moss protonemata and fern prothalli. They appear to be symbionts, a relationship already known for some species of the genus living on tracheophyte leaves, where they consume the methanol emitted from stomatal pores and supply growth-promoting phytohormones.



Figure 29. *Funaria hygrometrica*, substrate from which *Methylobacterium funariae* was described as a new species. Photo by James K. Lindsey, through Creative Commons.

Tani and Sahin (2013) named two new species *Methylobacterium haplocladii* (see Figure 6) and *Methylobacterium brachythecii* (see Figure 6) from bryophytes. These pink bacteria were isolated from *Haplocladium microphyllum* (Figure 30) and *Brachythecium plumosum* (Figure 31), respectively.



Figure 30. *Haplocladium microphyllum*, a species where a new species of *Methylobacterium* (*M. haplocladii*) was discovered. Photo by Bob Klips, with permission.



Figure 31. *Brachythecium plumosum* with capsules, a species where a new species of *Methylobacterium* (*M. brachythecii*) were discovered. Photo by Hermann Schachner, through Creative Commons.

Saumya *et al.* (2019) added to our knowledge by examining the bacterial flora of the mosses *Anoetangium clarum* (see Figure 32), *Atrichum undulatum* (Figure 33), and *Hyophila involuta* (Figure 34) on Mount Abu in India. Like the study by Koua *et al.* (2015) in Japan, they found the bacteria to belong mostly to the family **Methylobacteriaceae** and phylum **Bacillota**, with  **$\gamma$ -Proteobacteria** predominating. Genera that are most common in the various habitats of soil, near water, and on rocks are *Aeromonas* (**Pseudomonadota**; Figure 35), *Halobacillus* (**Bacillota**), *Pseudomonas* (**Pseudomonadota**; Figure 10), and *Raoultella* (Figure 36).



Figure 32. *Anoetangium compactum*; *Anoetangium clarum* in India supports mostly **Pseudomonadota** and **Bacillota**. Photo by Jan-Peter Frahm, with permission.





Figure 33. *Atrichum undulatum* in India supports mostly **Pseudomonadota** and **Bacillota**. Photo by Hermann Schachner, through Creative Commons.



Figure 34. *Hyophila involuta* in India supports mostly **Pseudomonadota** and **Bacillota**. Photo by Bob Klips, with permission.

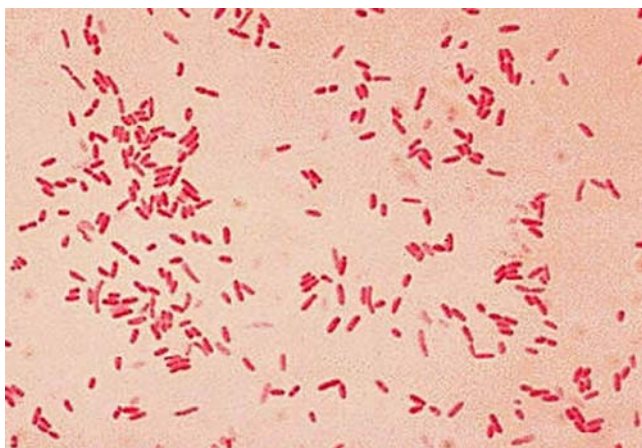


Figure 35. *Aeromonas hydrophila*, in one of most common bacterial genera on bryophytes in the various habitats of soil, near water, and on rocks in Japan. Photo by W. A. Clark, CDC, through public domain.

Alcaraz *et al.* (2018) noted that microbiomes influence plant establishment, development, nutrient acquisition, pathogen defense, and health. They compared the microbiomes of *Marchantia polymorpha* (Figure 37) and *Marchantia paleacea* (Figure 38) to the microbiomes on their soil substrates and to plants grown from gemmae

collected in the same populations of *Marchantia*. They identified *Bryobacter* (Acidobacteriota; Figure 39), *Lysobacter* (Pseudomonadota; Figure 40), *Methylobacterium* (Figure 6), *Paenibacillus* (Bacillota; Figure 41), *Pirellula* (Planctomycetes), *Rhizobium* (Pseudomonadota; Figure 42), and *Steroidobacter* (Pseudomonadota; Figure 43) associated with the *Marchantia*, genera that contribute to plant-growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression. They suggested that these *Marchantia* species could be used as surrogates for testing the roles of bacteria in plants.



Figure 36. *Raoultella planticola* culture, in one of most common bacterial genera on bryophytes in the various habitats of soil, near water, and on rocks in Japan. Photo by A. Doubt, through Creative Commons.



Figure 37. *Marchantia polymorpha* with gemmae, a species that is host to bacteria that contribute to plant growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression. Photo by Holger Casselmann, through Creative Commons.





Figure 38. *Marchantia paleacea*, a species that is host to bacteria that contribute to plant growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression. Photo by Naufal Urfi Dhiyaulhaq, through Creative Commons.

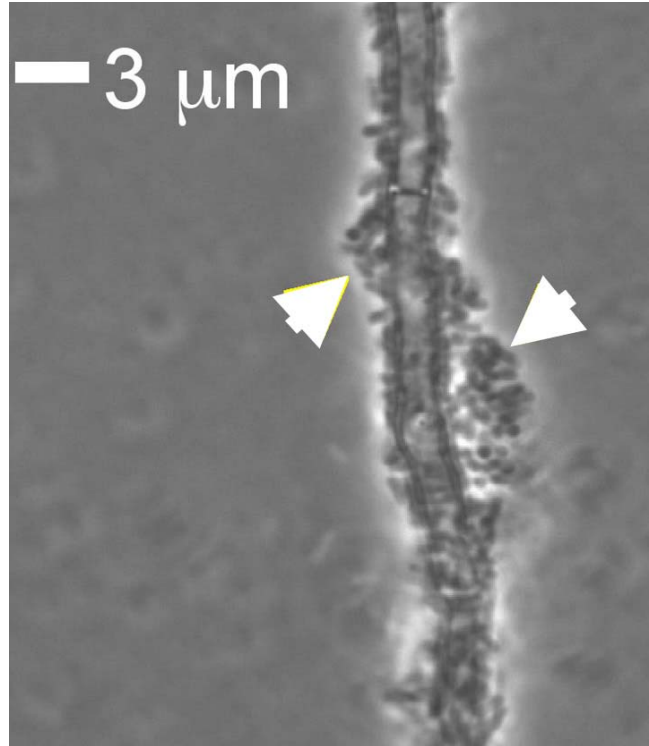


Figure 40. *Lysobacter*, a genus that contributes to plant-growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression in species of *Marchantia*. Photo through Creative Commons.

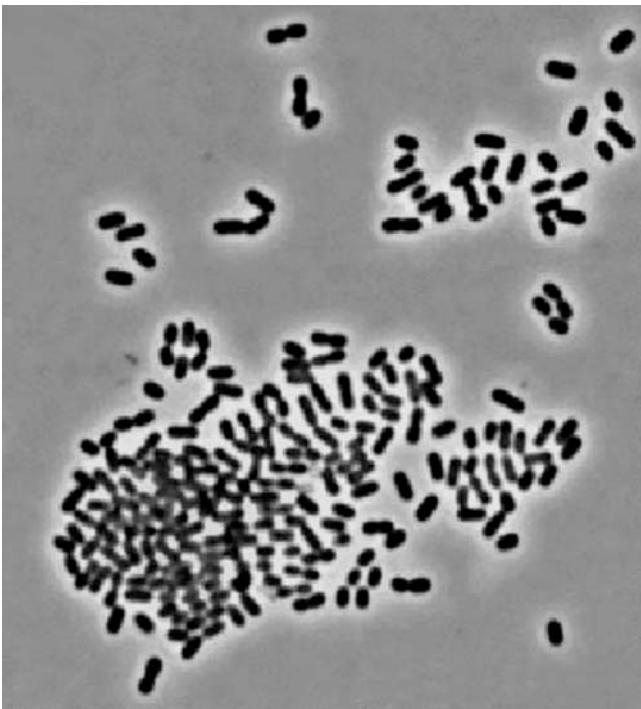


Figure 39. *Bryobacter aggregatus*, in a genus that contributes to plant growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression in species of *Marchantia*. Photo courtesy of the U.S. National Library of Medicine.



Figure 41. *Paenibacillus dendritiformis*, in a genus that contributes to plant growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression in species of *Marchantia*. Photo by Eshel Ben-Jacob, through Creative Commons.





Figure 42. *Rhizobium* nodules attached to roots of *Vigna unguiculata* (cowpea). *Rhizobium* species contribute to plant growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression in species of *Marchantia*. Photo by stdout, through Creative Commons.



Figure 44. *Marchantia inflexa*, a species that benefits from bacteria to improve acclimation to the local environment and may depend on them to create subtle differences in physiology and form between the sexes. Photo by Scott Zona, through Creative Commons.

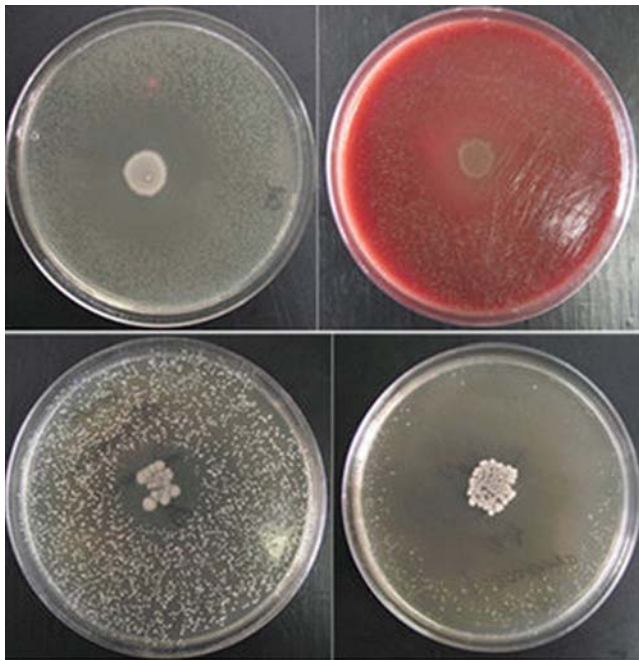


Figure 43. *Steroidobacter denitrificans* growth inhibition zones on various media; members of this genus contribute to plant growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression in species of *Marchantia*. Photo through Creative Commons.



Figure 45. *Marchantia inflexa* plants expressing female characters. Photo by Alan R. Franck, through Creative Commons.

Marks *et al.* (2018) compared the bacterial community of *Marchantia inflexa* (Figure 44-Figure 45) between sexes and among habitats. Using common garden conditions, they found that the bacterial community associated with the liverwort is abundant and diverse. The particular taxonomic assemblages of bacteria may serve functional roles that allow the liverworts to better acclimate to their local environment. Furthermore, the differences in communities on the two sexes of the plants may contribute to subtle differences in their physiology and form.

Aschenbrenner *et al.* (2017) compared communities associated with different substrata of bark, mosses, and lichens in Austria and revealed significant differences in community structures. The lichen microbial communities are less complex and less densely interconnected than the moss- and bark-associated communities. Generalists were mostly *Pseudomonadota*, with *Sphingomonas* (Figure 46) being the most abundant genus. The researchers suggested that the generalists benefitted each other and the community by maintaining a pool of species that were available to colonize new plants where they provided nitrogen fixation and other supporting functions. This sharing of hosts lends stability to the microbial community.





Figure 46. *Sphingomonas phyllosphaerae*, member of a generalist bacterial genus that can occur on bryophytes. Photo by Alan Rockefeller, through Creative Commons.



Figure 48. *Grimmia montana*, a species with high bacterial richness in a Chinese study, with **Pseudomonadota** and **Bacteroidota** being the most dominant phyla. Photo by Des Callaghan, through Creative Commons.

Tian and Li (2017) similarly found **Pseudomonadota** and **Bacteroidota** to be the most dominant phyla in their study of the mosses *Entodon compressus* (matrix under tree; Figure 47), *Grimmia montana* (exposed rock surface; Figure 48), and *Hygroamblystegium noterophilum* (stream bank; Figure 49) at the Beijing Songshan National Nature Reserve, China. The greatest species richness occurred on *Entodon compressus*, followed by *Grimmia montana* and *Hygroamblystegium noterophilum*, based on 16s rDNA libraries. On the other hand, the 16s rRNA libraries indicated that richness was of the order 73, 18, and 45, respectively. The **Pseudomonadota** comprised 33.7-86.1% of the communities and **Bacteroidota** 8.4-54.9% as the dominant phyla regardless of moss species. Nevertheless, the ratio and composition of the groups varied widely.



Figure 47. *Entodon compressus*, a species with the greatest bacterial richness in a Chinese study, with **Pseudomonadota** and **Bacteroidota** being the most dominant phyla. Photo by Martin Hutten, with permission.



Figure 49. *Hygroamblystegium noterophilum*, a species with less bacterial richness than *Entodon compressus* or *Grimmia montana* in a Chinese study, with **Pseudomonadota** and **Bacteroidota** being the most dominant phyla. Photo by Jean Faubert, with permission.

**Actinomycetota** and **Acidobacteriota** were abundant on *Entodon compressus* (Figure 47) (Tian & Li 2017). This moss supported a community of *Sphingomonas* (Figure 46), *Pseudonocardia* (**Actinomycetota**; Figure 50), *Bryobacter* (**Acidobacteriota**; Figure 39), *Flavisolibacter* (**Bacteroidota**), *Acidiphilium* (**Pseudomonadota**), and *Roseateles* (**Pseudomonadota**). *Sphingomonas* is tolerant of low temperatures and produces growth-promoting substances. *Pseudonocardia* has antibacterial activity. *Acidiphilium* is able to solubilize rock phosphates. *Roseateles* can degrade aliphatic and aliphatic-aromatic copolyesters. The researchers speculated that this bacterial community might be important in community dynamics in the organic matter associated with the *Entodon compressus*. Associated with *Grimmia montana* (Figure 48) they found *Rheinheimera* (**Pseudomonadota**; Figure 51), a genus that might be useful for the growth of this species on exposed rock with very little matrix by inhibiting the production of other microbes. This genus occurred in multiple locations and has antibiotic properties that might inhibit other bacteria.





Figure 50. *Pseudonocardia* on *Acromyrmex* worker, a bacterium cultured by the ant to protect fungus farms. This bacterium occurs on the moss *Entodon compressus*. Photo by João Pedro Sá Medeiros, through Creative Commons.

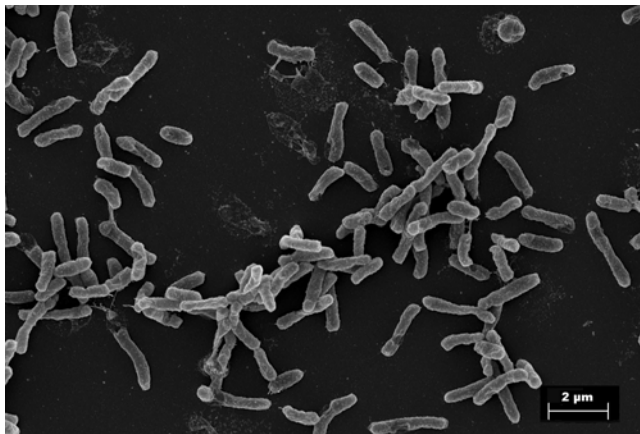


Figure 51. *Rheinheimera baltica* SEM; some members of this genus grow in association with *Grimmia montana* on bare rocks, where they are suspected of enhancing the moss growth by inhibiting other microbes. Photo by Manfred Rohde, through Creative Commons.

Saha *et al.* (2021) investigated the bacteria associated with the moss *Plagiomnium rostratum* (Figure 52). They found that the predominant bacterial species were members of the families **Bacillaceae** (**Bacillota**), **Enterobacteriaceae** (**Pseudomonadota**; Figure 14), **Lactobacillaceae** (**Bacillota**), **Moraxellaceae** (**Pseudomonadota**), and **Pseudomonadaceae** (**Pseudomonadota**). Many of the bacteria isolated were able to solubilize phosphates and scavenge nitrogen efficiently, as well as degrade starch, cellulose, and casein. They found that variation in the bacterial association was significantly correlated with total carbohydrate and phosphorus contents of the moss gametophytes.



Figure 52. *Plagiomnium rostratum*, a moss colonized mostly by members of the **Bacillota** and **Pseudomonadota**. Photo by Hermann Schachner, through Creative Commons.

## Effects on Bryophytes

The relationships between bacteria and bryophytes has been almost totally neglected (Jessica M. Nelson, Bryonet 22 April 2021). Recently a few researchers have begun to uncover exciting roles that these might play in the physiology of bryophytes. In sharp contrast, we are now learning about exciting interactions between these two groups of organisms.

Carella and Schornack (2018) described the relationship between bacteria and bryophytes as an association "with a strong and directed effort [by bacteria] to reprogram host cells [of bryophytes] in order to permit, promote and sustain microbial growth. In response to colonization, hosts accommodate or sequester invading microbes by activating a set of complex regulatory programs that initiate symbioses or bolster defenses."

Alvarez *et al.* (2016) found that the level of expression of antibacterial genes by the mosses were dependent on the developmental stage of the mosses. There was greater expression by the gametophore tissue than by the protonema tissue. Could these relate to habitat conditions at the time of development? Or is there an energy limitation on the protonema? Production of secondary compounds used for defense requires resources that compete with resources needed for growth and reproduction. Therefore, there is most likely a tradeoff, with the bryophyte optimizing its production of secondary compounds by producing them when they are needed most for the continuation of the species. On the other hand, having bacteria that produce defenses against the pathogenic bacteria in the association would be an important savings of resources.

## Symbiosis

There is limited direct evidence of symbiotic relationships between bryophytes and bacteria. The evidence that exists suggests that this is an area that warrants our attention. At the very least, the relationship does not seem to be neutral, with cases of protocoeoperation, commensalism, and antagonism, as well as symbiosis.



## Nitrogen Fixation

The nitrogen-fixing bacterial genus *Bradyrhizobium* (*Pseudomonadota*; Figure 1, Figure 53) forms a symbiotic connection with the adventitious roots of its host, *Acacia koa* (Figure 54) in Hawai'ian mesic forests. Leary *et al.* (2004) discovered that when these symbioses occur in mosses growing in the canopy, they form more and larger nodules than when associated with roots in soil.



Figure 53. *Bradyrhizobium* nodules with moss on *Acacia koa*. Photo courtesy of James Leary.



Figure 54. *Acacia koa*, a tree that benefits from mosses associated with its nitrogen-fixing *Bradyrhizobium* nodules. Photo by Forest and Kim Starr, through Creative Commons.

## Methylobacteria

The **methylobacteria** are a group of bacteria that are able to use methanol as their sole source of carbon and energy (Corpe & Basile 1982). They have been isolated from the surfaces of bryophytes. There is evidence that these pink, facultative methylotrophs are beneficial to the plants on which they grow. Evidence suggests this includes bryophytes.

Alcalde *et al.* (1996) demonstrated a little-known interaction between the moss *Bartramia* (Figure 55) and the genus *Methylobacterium* (Figure 6) in Spain, a relationship discussed elsewhere in this chapter for peatland habitats. Bryophytes and tracheophytes have

pectin in their cells walls, causing them to emit methanol. The pink-pigmented *Methylobacterium* (Figure 6) species are able to colonize leaf surfaces and use the methanol as their only source of carbon and energy (see also Raghoebarsing *et al.* 2005; Liu *et al.* 2014).

Kutschera (2007) found that the tracheophytes failed to respond to the relationship. However, development of both the mosses and liverworts in the study was affected. Organ development in moss protonemata and in liverwort thalli was "considerably" enhanced. *Methylobacterium* secretes both cytokinins and auxins that can initiate or control developmental stages. This seems only to affect haploid stages (gametophytes) and the interaction has been lost in tracheophytes that are apparently able to sufficiently produce and control their own growth hormones.

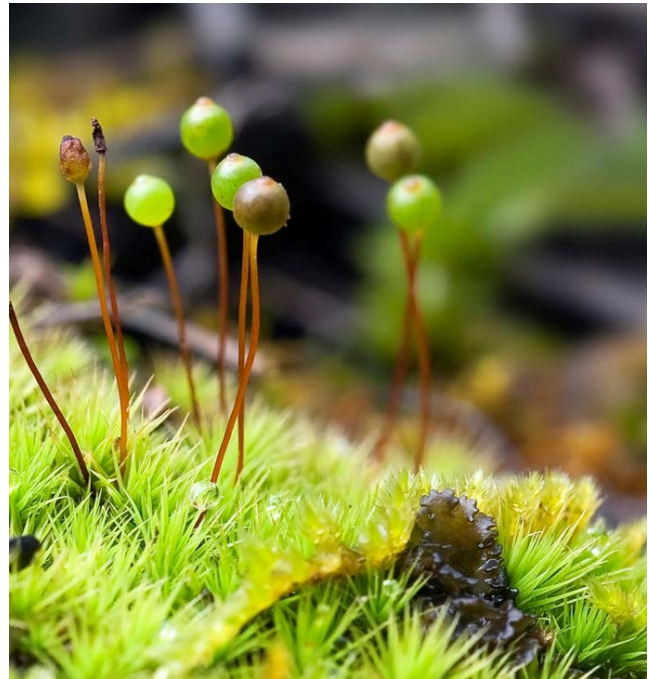


Figure 55. *Bartramia stricta* with capsules; a species of *Methylobacterium* in Spain uses methanol as its only source of carbon and energy. This is emitted by the moss and provides needed carbon for the *Methylobacterium*, which in turn releases CO<sub>2</sub> used by the moss. Photo by John Game, through Creative Commons.

In bryophytes, *Methylobacterium* (Figure 6) enhances cell growth (Kutschera *et al.* 2007). Bacteria isolated from the upper surface of the thalli of *Marchantia polymorpha* (Figure 37) proved to be an undescribed species of *Methylobacterium*, now known as *Methylobacterium marchantiae* (Schauer *et al.* 2011; see Figure 6). This bacterium stimulates the surface expansion of isolated gemmae (Figure 37, Figure 56) from *M. polymorpha* by about 350% (Kutschera *et al.* 2007)! In water suspension, the *Methylobacterium marchantiae* from the liverwort forms dense clusters of up to 600 cells. But when *Methylobacterium mesophilicum*, a tracheophyte associate, is cultured in water, only single cells are formed. Kutschera and coworkers suggested that the clusters on the liverwort inhabitant were an adaptation to surviving on the liverwort when it underwent desiccation in its natural habitat.





Figure 56. *Marchantia polymorpha* gemma. The bacterium *Methylobacterium marchantiae* stimulates the surface expansion of such isolated gemmae. Photo by Des Callaghan, through Creative Commons.

Kutschera and Koopmann (2005) discovered that the thallose liverworts *Marchantia polymorpha* (Figure 37) and *Lunularia cruciata* (Figure 57) serve as host plants for the genus *Methylobacterium* (Figure 6) that secretes phytohormones on the surfaces of the thalli. These hormones promote the growth of isolated gemmae (Figure 56) on agar and appear to be a necessary component for the completion of the life cycle. When bryophytes first evolved, it appears that they depended on external sources such as bacteria for critical factors in their life cycles. They spent their evolutionary capital developing numerous secondary compounds so that they could survive the bacteria fungi, protozoa, and herbivores that threatened their existence.



Figure 57. *Lunularia cruciata* showing gemmae that respond to hormones secreted by *Methylobacterium*. Photo by Hermann Schachner, through Creative Commons.

Tian and Li (2017) identified the dominant methylamine-utilizing bacteria from *Hygroamblystegium noterophilum* (Figure 49) as *Methylotenera*, *Methyloversatilis*, and *Tepidimonas*. These genera contribute primarily to denitrification and methanol metabolism.

Hornschuh *et al.* (2002) found that bacteria were numerous on the leaf surfaces of moss *Funaria hygrometrica* (Figure 29). In particular, they occurred in

the grooves between adjacent lamina cells (Figure 58). Isolated strains of *Methylobacterium mesophilicum* (see Figure 6) and *Methylobacterium* sp. elicited the same response as cytokinin application on protonemal bud formation (Figure 59) and promoted growth of the protonemal filaments. This suggests that these bacteria have an important role in the development of *Funaria hygrometrica*.

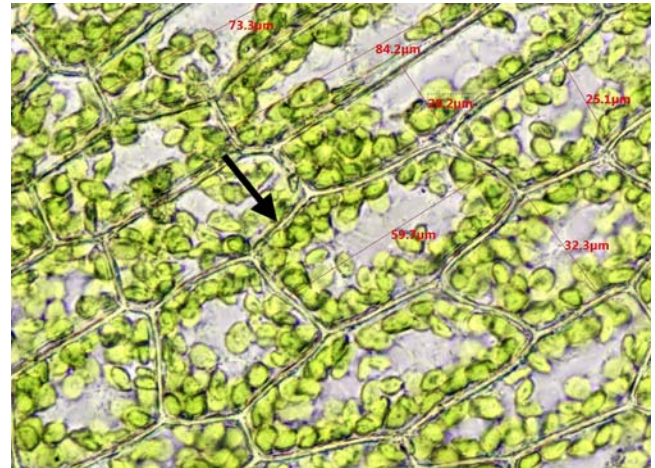


Figure 58. *Funaria hygrometrica* leaf cells; arrow indicates groove between two adjacent lamina cells where bacteria often grow. Photo by Claire Halpin, with permission.

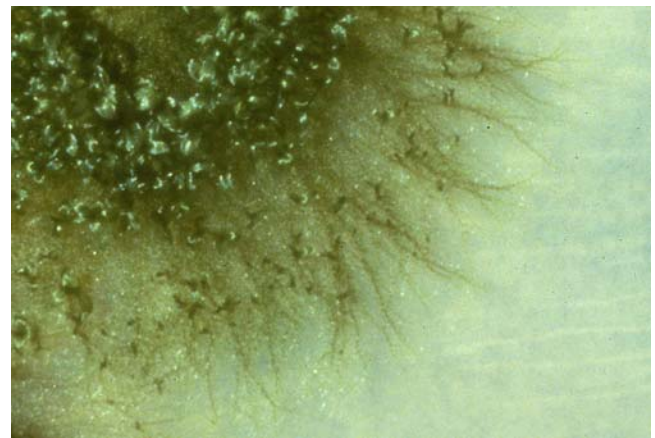


Figure 59. *Funaria hygrometrica* cultures with young gametophores and gametophore buds near the ends of the protonemata. *Methylobacterium* elicits a cytokinin type of response in the growth and bud formation of the protonema. Photo by Janice Glime.

Schauer and Kutschera (2011) further investigated the bacterium now known as *Methylobacterium funariae* (see Figure 6) isolated from *Funaria hygrometrica* (Figure 29). These bacteria provide cytokinins and auxins to the moss associates. In the association, methanol is emitted by the mosses and used by the bacteria as their carbon source. Schauer and Kutschera suggested that amino acids leached from the bryophytes might be important as sources of carbon and nitrogen for the bacteria.

### CO<sub>2</sub> Source

One of the first considerations regarding bryophyte interactions with bacteria was that bacteria provide a source



of CO<sub>2</sub> for the bryophytes, particularly in aquatic habitats. Wetzel *et al.* (1985) noted that algae and aquatic plants are rapidly limited by low availability of CO<sub>2</sub> even at low pH in the range of 4-6. They found that 25-40% of the carbon fixed by leaves can originate from the sediments. When more CO<sub>2</sub> becomes available in the rhizosphere sediments, the reliance on CO<sub>2</sub> diminishes.

In the remote location of Antarctica, Tarnawski *et al.* (1992) noticed differences in growth of the moss *Schistidium chrysoneurum* (Figure 60). This moss grows as turf in wet locations and as cushions at relatively dry sites. Tarnawski and coworkers discovered that the CO<sub>2</sub> concentrations within these two communities differed "substantially." At the beginning of the growing season, both communities had the same CO<sub>2</sub> concentrations of about 350 ppm. But in the turf, the CO<sub>2</sub> levels rose tenfold during the growing season while those in the cushions changed little. This provided ideal growing conditions in the turf. The researchers attributed the higher CO<sub>2</sub> levels to respiration of rhizoids and heterotrophic communities, including the bacterial component.



Figure 60. *Schistidium chrysoneurum* in Antarctica, a species that benefits from the CO<sub>2</sub> produced by bacteria. Photo by Sharon Robinson, with permission.

In another example a surprisingly large colony of *Fontinalis cf. novae-angliae* (Figure 61-Figure 62) was discovered on the floor of Yellowstone Lake, a 119-m-deep lake in Yellowstone National Park, USA, at 2,357 m asl (Lovalvo *et al.* 2010). Due to its elevation and location, the lake averages a temperature of 5°C. We would expect that the attenuation of light and the cold temperatures at that depth would discourage the growth of any photosynthetic organism other than some highly adapted algae. In the lake, the mosses were associated with geothermal vents where the water was supersaturated with CO<sub>2</sub>. This situation illustrates the ability of high CO<sub>2</sub> levels to enhance photosynthesis in otherwise limiting conditions. Thus, we should look for aquatic mosses at depths where heterotrophic bacteria benefit from organic sediments and release respiratory CO<sub>2</sub> that is available to the aquatic bryophytes.

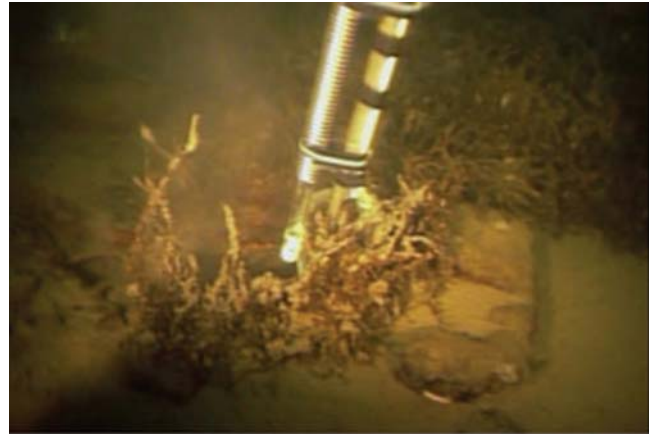


Figure 61. *Fontinalis cf. novae-angliae* from Yellowstone Lake geothermal vent, where bacterial respiration most likely contributes to its needed CO<sub>2</sub>. Photo from Lovalvo *et al.* 2010.



Figure 62. *Fontinalis novae-angliae* habitat in a stream; bacteria associated with the moss most likely contribute CO<sub>2</sub> for photosynthesis by the moss. Photo by Janice Glime.

Gimeno *et al.* (2017) suggested that bacterial partners could contribute to carbonyl sulphide production (COS) in bryophytes. Uptake of COS, a surrogate for measuring photosynthesis, could be significant in bryophyte cells at night, as suggested by their experiments, because bryophytes are able to take in COS in the dark, using the light-independent carbonic anhydrase, not relying on light to open stomata as is the case for tracheophytes. Carbonyl sulfide is an intermediate between carbon dioxide and carbon disulfide (Wikipedia 2022). With sufficient humidity or water in association with bases, carbonyl sulfide decomposes to carbon dioxide and hydrogen sulfide. Could this help to account for the bryophytes that occur in highly alkaline waters? It is unclear if the bryophytes can benefit the bacteria at night, but in the daytime they could provide O<sub>2</sub>.

## Growth Hormones

One of the important discoveries in the bryophyte-bacteria relationship is that bacteria can provide hormones that are necessary for the development of bryophytes through the life cycle. Researchers have discovered that optimal growth conditions, including development and reproduction, often require interactions with



microorganisms in a parasitic, mutualistic, or protocoeperative relationship (Spiess *et al.* 1984a, 2019).

### Bud Induction

I suspected such a relationship between bacteria and protonemal development in the 1980's when I cultured *Fontinalis squamosa* (Figure 63-Figure 64) from spores (Glime & Knoop 1986). I cultured these in the lab of Martin Bopp in Germany and had to abandon them to return to my responsibilities in the USA. My colleague, Bernd Knoop, continued to watch the cultures until they became contaminated, at which time they were discarded. But he reported to me that the only buds (see Figure 65) on my cultures were on the contaminated cultures. That suggested to me that my sterile cultures needed something that was produced by partner organisms in nature. Ares *et al.* likewise concluded that the developmental differences between the axenic cultures of *Fontinalis antipyretica* (Figure 66) and those contaminated with bacteria (or fungi) were likely to be due to interaction with the contaminants.



Figure 63. *Fontinalis squamosa* in stream at Cwm Idwal National Nature Reserve, Wales. This species seems to require bacterial hormones to complete its development. Photo by Janice Glime.



Figure 64. *Fontinalis squamosa* protonema; this species seems to need hormones from bacteria to advance to the bud stage. Photo by Janice Glime.



Figure 65. Moss protonema with young bud. Development of this stage often seems to require hormones from bacteria. Photo by Chris Lobban, with permission.



Figure 66. *Fontinalis antipyretica*, a species that seems to gain developmental benefits from microbes. Photo by Misha Ignatov, with permission.

As we now have observed in many other axenic cultures of bryophytes, the protonemata of *Hyophila involuta* (Figure 34) failed to produce buds on basal Knop's + Nitsch's minor salts (Rahbar & Chopra 1982). Furthermore, addition of auxins, gibberellic acid, abscisic acid, chelates, vitamin B<sub>12</sub>, activated charcoal, coconut milk, and altered hydration, pH, temperature, light intensity and duration all failed to stimulate bud formation. Cytokinins could initiate multicellular gemmae on the protonemata, but failed to initiate buds. Only the interaction of IAA with either kinetin or DMAAP stimulated formation of buds and normal gametophore development. Such observations suggest that in nature some exogenous source, perhaps from bacteria or fungi, contributes the hormones necessary to initiate the next developmental stage.

Reutter *et al.* (1998) found that application of cytokinins to *Physcomitrium patens* (syn. = *Physcomitrella patens*; Figure 67) cultures enhances bud formation but fails to stimulate the subsequent gametophore development. Most of the cytokinin and auxin occur in extracellular pools and appear to be involved in hormone transport in mosses. Gonneau *et al.* (2001)



further demonstrated that development in *Physcomitrium patens* is regulated by environmental signals and hormones. Cytokinins are required to give rise to the leafy gametophore, but it appears to be regulated to different concentrations in the bud stage compared to elongation of the gametophore.



Figure 67. *Physcomitrium patens*; AHLs from bacteria promote spore germination in this moss. Photo by Hugues Tinguy, with permission.

My suspicion of bacterial hormone contributions was influenced by the early research of Loretta Spiess and her coworkers. They were able to demonstrate that the bacterium *Agrobacterium tumefaciens* (Figure 68) influenced the development of the epiphytic moss *Pylaisiella selwynii* (Figure 69), including initiation of gametophore buds more quickly (Spiess *et al.* 1971). After 35 days, mosses cultured axenically exhibited only 0-24% gametophore formation, whereas those inoculated with *A. tumefaciens* had at least 96% gametophore formation. Bacterial-assisted cultures also produced 4-6 gametophores per culture, compared to 1 in the absence of the bacteria. The supernatant from the cultures did not cause any changes in bud production.

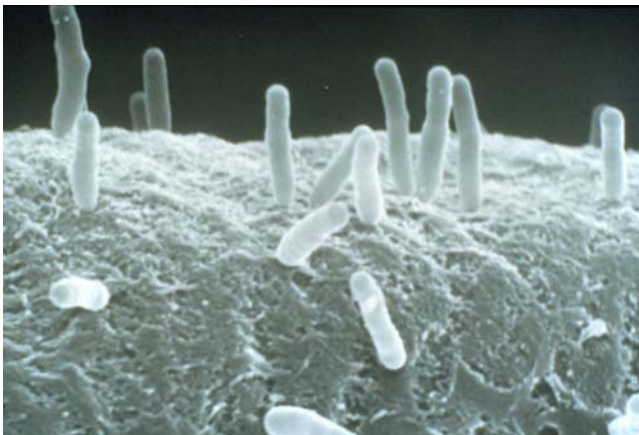


Figure 68. *Agrobacterium tumefaciens*, a species that provides hormones needed for the development of *Pylaisiella selwynii*. Photo by Martha Hawes, University of Arizona through NSF public domain.



Figure 69. *Pylaisiella selwynii*, a moss that has a hormonal benefit from the bacterium *Agrobacterium tumefaciens*. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Spiess *et al.* (1972) explored the possible influence of bacteria by testing the effects of various hormones on *Pylaisiella selwynii* (Figure 69). They found that indoleacetic acid (IAA) and ethrel increased bud formation at a narrow concentration range. But bud formation responded well at various concentrations of cytokinins. Nevertheless, the cytokinin-induced buds failed to develop into normal gametophores. This is not surprising because Bopp and Jacob (1986) later found that in the moss *Funaria hygrometrica* (Figure 29) the concentration of cytokinin that effects branching of caulonemata requires pico-molar concentrations, whereas bud formation requires micro-molar concentrations.

More encouraging for Spiess *et al.* (1972) was the fact that octopine, lysopine, and octopinic acid from crown-gall tumors increased *Pylaisiella selwynii* (Figure 69) bud formation at  $10^{-3}$  M. In particular, lysopine stimulated the formation of buds that developed into typical gametophores. However, octopine initiated the formation of gemma-like structures, but no gametophores. Culturing with l-arginine from octopine and l-lysine from lysopine failed to induce gametophore formation.  $\gamma$ -guanidinobutyric acid induced bud formation at  $10^{-3}$  M concentrations; the buds produced highly abnormal gametophores. Reminiscent of the ineffectual influence of the supernatant, Spiess *et al.* (1976) found that physical contact was necessary for the bacteria to be effective in production of gametophores.

Whately and Spiess (1977) demonstrated that LPS (lipopolysaccharide) from *Agrobacterium tumefaciens* (*Pseudomonadota*; Figure 68) inhibited gametophore development by preventing the bacterium from binding, providing further evidence that direct contact was needed between the moss and the bacterium. This effect is apparently only effective for a short time; if the LPS was added 24 hours after the addition of the bacterial cells, it had no effect in reducing the development of the gametophore.



Protonemal growth of *Pylaisiella selwynii* (Figure 69) was slightly accelerated by cAMP (Spiess 1979). IAA ( $10^{-6}$  M) alone, or with cAMP, inhibited protonemal elongation but when added at  $10^{-12}$  M it increased filament growth, demonstrating the importance of the concentration. When adenosine and guanosine were added together (depending on the ratio), they caused a marked increase in rapidly elongating normal gametophores.

After ten years of study with *Agrobacterium* (Figure 68), Spiess *et al.* (1981a) still could not assign the bacterial isolates from three other species of mosses and *Pylaisiella selwynii* (Figure 69) from another location to the genus *Agrobacterium*. Many of these isolates elicited developmental changes in the protonemata of *Pylaisiella selwynii* (Figure 69) that were similar to those of the *Agrobacterium*. In any case, it was becoming clear that in nature bacteria can influence the developmental stages of mosses.

Spiess *et al.* (1981b) again pursued the effects of octopine and cytokinin on the growth and gametophore formation of *Pylaisiella selwynii* (Figure 69). Octopine is an unusual amino acid, but it occurs in crown gall tumors. In combination with cytokinin it increased the number of gametophores and decreased the time required for them to develop. This effect was similar to that seen with *Agrobacterium* (Figure 68) in *Pylaisiella selwynii* cultures. But concentration was important. More common amino acids alone or in combination with auxins or cytokinins generally had a neutral effect on the moss development.

There is an interesting inhibitory action by the cell walls of *Pylaisiella selwynii* (Figure 69). Cell walls of several dicots, but not of tested monocots, inhibited the induction of buds and gametophore development by *Agrobacterium tumefaciens* (Figure 68) (Spiess *et al.* 1984b). Both pectin and polygalacturonate were inhibitory. Protonemal cell walls inhibited gametophore induction; gametophores were less inhibitory. But cell walls from the moss *Polytrichum commune* (Figure 24) protonema and gametophores caused little inhibition. On the other hand, *Agrobacterium* is ineffective in increasing bud formation in *Polytrichum commune*. If the *Polytrichum* protonemata or gametophore cell walls are treated with pectinesterase, they do inhibit the developmental stimulation of *Agrobacterium* on *Pylaisiella selwynii* and pectinesterase increases the inhibitory effect by *Pylaisiella* gametophore cell walls. Conversely, pectinesterase treatment of the *Polytrichum* protonema makes it more sensitive to the *Agrobacterium*, causing increased bud and gametophore formation. Spiess and coworkers reasoned that the bacteria require suitable adherence sites and that the addition of the pectinesterase made these sites available in *Polytrichum*.

One effect of at least some bryophytes on *Agrobacterium tumefaciens* (Figure 68) is the ability to induce the expression of its virulence gene (Primich-Zachwieja & Minocha 1991). This was evident by the  $\beta$ -galactosidase activity in the bacteria.

While Spiess and coworkers were attempting to understand the relationships of bacteria with *Pylaisiella selwynii* (Figure 69), Chopra and Vashistha (1990) explored the effect of auxins and antiauxins on the shoot bud induction and growth form of the moss *Bryum atrovirens* (Figure 70). In culture, various auxins induced buds on the protonemata, whereas without these added

hormones the sterile culture conditions were not conducive to bud formation. Again, concentration was important, with higher levels causing adverse effects on the morphology.



Figure 70. *Bryum atrovirens*; various auxins induced buds on the protonemata, whereas without these added hormones buds were absent; bacteria most likely supply these auxins in nature. Photo by Jan-Peter Frahm, with permission.

The study of hormones and their effects on bryophytes has been largely confined to auxins and cytokinins (Sabovljević *et al.* 2014). Gibberellic acid (GA) has been mostly ignored, with investigations suggesting that it did not evolve its interaction with GID1-DELLA until after bryophytes diverged from other land plants (Yasumura *et al.* 2007). ABA and its sister compound lunularic acid have been studied somewhat extensively (Decker *et al.* 2006).

Chopra and Dhingra-Babbar (1984) also found that indoleacetic acid (IAA), gibberellic acid, abscisic acid, chelates, salicylic acid, and altered temperature, pH, agar, sucrose levels, light levels, and photoperiod do not induce buds in the moss *Trematodon brevicalyx* (see Figure 71). Only cytokinins elicited a bud response in sterile cultures. In fact, even at concentrations of cytokinins that induced buds, varying concentrations of IAA reduced the number of buds considerably.



Figure 71. *Trematodon longicollis* on a wet roadside bank; *Trematodon brevicalyx* requires cytokinins to induce bud formation in culture, hormones most likely supplied by bacteria in nature. Photo by Michael Lüth, with permission.



Yasumura *et al.* (2007) demonstrated a lack of GA production in *Physcomitrium patens* and suggested the pathway to its production arose after the bryophyte lineage. Nevertheless, gibberellic acid, a known product of bacteria (MacMillan 2002; Yamaguchi 2008) has a positive effect on morphogenesis in *Bryum argenteum* (Figure 72) (Sabovljević *et al.* 2010) and interferes with gravitropism in *Ceratodon purpureus* (Figure 73) (Chaban *et al.* 1999). Since bacteria that inhabit plants are able to produce gibberellic acid (Katznelson & Cole 1965; MacMillan 2002; Karakoç & Aksöz 2006; Zhang *et al.* 2012; Ambawade & Pathade 2015; Desai 2017), this interaction should be explored with bryophytes *in situ* and in the lab.



Figure 72. *Bryum argenteum*, a moss species that is positively affected by gibberellic acid. In nature this is probably supplied by bacteria and other microorganisms. Photo by Tushar Wankhede, with permission.



Figure 73. *Ceratodon purpureus*, a species in which gibberellic acid interferes with gravitropism. Photo by Janice Glime.

If mosses respond to different concentrations in different ways, how do bacterial levels coordinate the developmental stages? Do the bacteria respond to environmental signals so that protonemata branch while the bacteria are at low numbers (that would give bacteria more cover and hold moisture better), then the bryophytes

develop gametophores when the bacterial numbers increase (that would ensure a large colony of mosses that can help to conserve moisture within the colony)? Do differences in developmental responses occur among bryophyte species? If so, how important are the bacteria species in determining the success of specific bryophyte species in particular habitats?

## Growth

The moisture-loving leafy liverwort *Scapania nemorea* (Figure 74) has a regular association with the bacterium *Pseudomonas extorquens* (see Figure 10) (Basile *et al.* 1969). When *S. nemorea* gametophytes were inoculated with this bacterium in culture, they grew larger and reached reproductive maturity more quickly than those cultures without the bacteria. It is likely that this stimulus occurs in nature as well.



Figure 74. *Scapania nemorea* with gemmae, a species with a regular positive association with the bacterium *Pseudomonas extorquens*. Photo by Blanka Aguero, through Creative Commons.

Tani *et al.* (2011) explored *Racomitrium japonicum* (Figure 75) with the intent of increasing its growth rate for culture as a green-roof plant. They isolated *Pseudomonas* (Figure 10), *Rhodococcus* (Actinomycetota; Figure 76), and *Duganella* (Pseudomonadota) species from hydroponic culture of the moss. The researchers characterized these bacteria by their plant interactions such as auxin production, siderophores (molecules that bind and transport iron in microorganisms), or hydrogen cyanate, growth in absence of added nitrogen source, calcium phosphate solubilization, utilization of sugars, polymers, or aliphatic compounds, and antifungal activity. Such activities cause the bacteria to stabilize production and enhance the growth of *Racomitrium japonicum*.





Figure 75. *Racomitrium japonicum*; environmental bacteria (especially *Duganella*, *Pseudomonas*, and *Rhodococcus*) stabilize production and enhance the growth of *Racomitrium japonicum*. Photo from Digital Museum, Hiroshima University, with permission.



Figure 76. *Rhodococcus*, a bacterium that enhances growth and production of *Racomitrium japonicum*. Photo by David Berd, CDC, through public domain.

### Rhizoids

Sheldrake (1971) determined that the concentrations of auxins in the soil were in the same range as those known to stimulate the formation of rhizoids in liverworts. Sheldrake further considered that the greatest concentration of auxins would occur in areas with the highest nutrient levels. This mechanism would cause the bryophytes to produce the most rhizoids in microhabitats with the highest concentrations of nutrients. Sheldrake concluded that the bryophytes did not produce auxins and that they depended on the environment to supply them. Hence, the bacteria could provide an important role in signalling environmental conditions to the bryophytes. This increased production of bryophyte rhizoids could be beneficial in high-nutrient environments that would also increase competition from other plant species.

Khan *et al.* (1997) found that rhizoids of mosses could also stimulate the growth of bacteria. This was particularly

true for the bacterium *Bacillus* (Figure 9). Where do these mutual stimulation partnerships end?

### Quorum Sensing

It is important to realize that bacteria do not live as solitary cells, but that they require the coordination of a colony with intercellular communication that permits them to adjust to changing environmental conditions (Whitehead *et al.* 2001). This communication, as we might expect, is through chemical signals. These signals are dependent on cell density and growth phase.

Bacteria use **quorum sensing** as a way of monitoring their population density and interacting with their environment (Vesty *et al.* 2020). Quorum sensing requires intercellular signalling mechanisms (ISMs) that serve as a means of recognizing cell density (Whitehead *et al.* 2001). In the environment, the expression of virulence depends on the synthesis of and response to diffusible signalling metabolites (Manefield & Turner 2002). Thus far, only the **Pseudomonadota** are known to produce the necessary AHL (N-acyl-L-homoserine lactone) compounds used for signalling, thus limiting the availability of such signalling. This may account for the preponderance of the **Pseudomonadota** in association with bryophytes. However, widespread testing of signalling among bacteria and to bryophytes is lacking. For example, <1% of all bacteria that are present in any environment can be cultured in the lab using standard media, so many more AHL-producing bacteria are possible (Vesty *et al.* 2020). Recent DNA techniques may help us to elucidate these bacteria.

As Whitehead *et al.* 2001 suggested, Williams *et al.* (2007) found that bacteria associated with bryophytes, instead of being the passive autonomous organisms we thought, are highly communicative. As the population density increases, the production of quorum sensing molecules also increases, increasing their presence in the external environment. Quorum sensing enables a bacterial population to achieve a co-operative response that improves access to nutrients or specific environmental niches, promotes collective defense against other competitor prokaryotic or eukaryotic defense mechanisms, and facilitates differentiation into forms that promote survival by making the cells better able to combat environmental threats. Quorum sensing can be exploited or inactivated by both plants and mammals, and it appears that bryophytes are among the users of this phenomenon.

### Spore Germination

Among the Gram-negative bacteria, the quorum sensing molecules are N-acylhomoserine lactones (AHLs) (Vesty *et al.* 2020). These AHLs can affect the spore germination of the moss *Physcomitrium patens* (Figure 67). AHLs promote this spore germination at sub-micromolar concentrations but inhibit spore germination at concentrations above 1  $\mu$ M. Even the sporophytes of some wild isolates of *Physcomitrium patens* are associated with AHL-producing bacteria. Many of the *Pseudomonas* (Figure 10) isolates, most of the *Serratia* (Figure 8) isolates, and one of the *Aeromonas* (Figure 77) isolates, all known from bryophytes, produced AHLs in their study. Furthermore, there are many bacteria that thus far have not been cultured, so there could be many additional sources of AHL's in the bryophyte habitats.



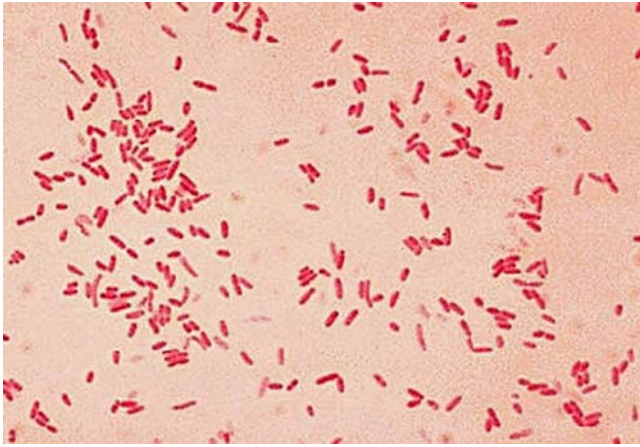


Figure 77. *Aeromonas hydrophila*, a bacterium that produces AHLs as signalling compounds. Photo by W. A. Clark, CDC, through public domain.

Vesty *et al.* (2016) concluded that endogenous hormone signalling networks that control germination of spores and seeds as environmental responses may have evolved independently in spores and seeds. Such parallel evolution is a testimony to the importance of the relationship.

### Vitamins

Algae use **vitamin B<sub>12</sub>** that is manufactured by bacteria, a symbiotic need generated by the lack of B<sub>12</sub>-dependent enzymes in algae (Croft *et al.* 2005). Bryophytes likewise obtain vitamin B<sub>12</sub> from bacteria (Basile *et al.* 1985), although it does not seem to have a direct role. Its presence in bryophyte-associated bacteria, however, could be important for animals feeding there, particularly large herbivores that use bryophytes as emergency food. Growth of *Lioclaena lanceolata* (Figure 78) and *Gymnocolea inflata* (Figure 79) was significantly stimulated by the pink facultative methylotrophic bacteria that both synthesize and accumulate vitamin B<sub>12</sub>. These bacteria commonly associate with bryophytes, but the physiological role of vitamin B<sub>12</sub> is elusive (Marsten 1952).



Figure 78. *Lioclaena lanceolata*, a species that is stimulated by the pink facultative methylotrophic bacteria that both synthesize and accumulate Vitamin B<sub>12</sub>. Photo by Bob Klips, with permission.



Figure 79. *Gymnocolea inflata*, a liverwort species that is stimulated by the pink facultative methylotrophic bacteria that both synthesize and accumulate Vitamin B<sub>12</sub>. Photo by Michael Lüth, with permission.

### Water Relations

Could bacteria help bryophytes in their recovery from desiccation? Or are they a threat to be reckoned with?

Minibayeva and Beckett (2001) suggested that the oxidative burst seen upon rehydration in a hornwort (*Anthoceros natalensis* – Figure 80) and two thalloid liverworts [*Dumortiera hirsuta* (Figure 81), *Pellia epiphylla* (Figure 82)] is actually a defense mechanism against pathogenic fungi and bacteria. Li *et al.* (2010) found a similar response to both biotic and abiotic stresses in *Dumortiera hirsuta*. When bryophytes desiccate, their membranes become leaky. When they rehydrate, bacteria and fungi can enter the leaky cells as the water rehydrates them (Minibayeva & Beckett 2001). Hence the oxidative burst can help to prevent those pathogens from damaging the cells of the bryophyte. It is interesting that mosses and at least some leafy liverworts tested lacked the oxidative burst and its absence may be related to their desiccation tolerance. We need experiments and observations to determine how well the oxidative burst correlates with desiccation tolerance, and is it needed more in those with higher moisture requirements?



Figure 80. *Anthoceros* sp. with capsules; *Anthoceros natalensis* seems to use oxidative burst seen upon rehydration as a defense against bacteria. Photo from USFWS, through public domain.





Figure 81. *Dumortiera hirsuta*; a liverwort that seems to use the oxidative burst seen upon rehydration as defense against bacteria. Photo by Shyamal L., through Creative Commons.



Figure 82. *Peltia epiphylla*; this liverwort seems to use the oxidative burst seen upon rehydration as defense against bacteria. Photo by Valentin Hamon, through Creative Commons.

Bacteria can confound measurements of productivity in bryophytes. From an ecosystem point of view, it may be legitimate to express the productivity of the bryophyte-periphyton association, but from a physiological perspective of the bryophyte alone, this is not acceptable. Gupta (1977) noted that following desiccation in the mosses *Dicranella palustris* (Figure 83), *Mnium hornum* (Figure 84), and *Syntrichia ruralis* (Figure 85), and the liverworts *Porella platyphylla* (Figure 82) and *Scapania undulata* (Figure 86) the external water collected from them after 22 hours had large numbers of microorganisms. The burst of respiration following rehydration was due to these microorganisms. But does this respiratory activity indicate damage to the bryophytes, providing leaked carbohydrates to the bacteria, or could it be a benefit by providing additional CO<sub>2</sub> for photosynthesis?

Do bacteria help in the uptake of nutrients in bryophytes? For the bean, *Phaseolus vulgaris*, 42 hours after 10 nM homoserine lactone (HL) was supplied to roots the transpiration and stomatal conductance increased significantly. Although the experiments were done with tracheophytes, the same effect could occur with bryophytes, especially those that form cushions. They lack

stomata, but transpiration still occurs from the cushion. Joseph and Phillips (2003) considered the bacteria in the soil to have a role in plant water and nutrient relations. As water diffuses from the plant to the atmosphere, the action helps to move diffusion-limited nutrients such as phosphorus from the soil to the plant and also to the microbes.



Figure 83. *Dicranella palustris*, a species that has large numbers of microorganisms in its external water, causing a burst of respiration upon rehydration of the moss. Photo by Andrew Hodgson, with permission.



Figure 84. *Mnium hornum*, a species that has large numbers of microorganisms in its external water, causing a burst of respiration upon rehydration of the moss. Photo by Bob Klips, with permission.



Figure 85. *Syntrichia ruralis*, a species that has large numbers of microorganisms in its external water, causing a burst of respiration upon rehydration of the moss. Photo by Bob Klips, with permission.





Figure 86. *Scapania undulata*, a liverwort species that has large numbers of microorganisms in its external water, causing a burst of respiration upon rehydration. Photo by Hermann Schachner, through Creative Commons.

By contrast, Krochko *et al.* (1978) found that respiration in the semiaquatic moss *Cratoneuron filicinum* (Figure 87) does not occur upon rehydration following rapid drying. They, too, cautioned that contamination by bacteria could cause false readings of the respiration by the moss.



Figure 87. *Cratoneuron filicinum*, a moss that does not exhibit a rapid respiration following rehydration. Photo by Claire Halpin, with permission.

## Freezing Protection

Liquid pure water does not freeze at 0°C, but requires the temperature to drop to -38°C before it freezes, and even lower in very small samples (Moffett 2015). But water does not occur in the ecosystem in its pure state. Instead it has many nucleating materials, including bacteria, that permit it to crystallize at a temperature near 0°C. In fact, it is the bacteria that permit it to freeze at the highest temperatures. The **ice nucleation** bacteria seem to be limited to a small number of plant pathogens that use specific proteins to cause freezing. The resulting damage permits them to gain nutrients from the plants. Moffett showed that ice nucleation is likewise an active process in both mosses and liverworts. In fact, those tested harbor 106-107 g L<sup>-1</sup>, an order of magnitude greater than that

known for lichens. But Moffett failed to find more than a few bacteria on the surfaces of mosses cultured on selective media, thus concluding that ice nucleation activity is unlikely to be caused by surface bacteria. But there seem to be many bacteria that have never been cultured because we don't know their requirements (Vesty *et al.* 2020). Could it be that tiny nucleating bacteria are present, but not yet detected by traditional methodology?

When ice forms on bryophytes, it grows at the expense of the bryophyte by pulling water from the cells or scavenging it from the surface. This is a particular problem for those species that are dependent on fog, dew, and cloudwater. Moffett *et al.* (2009a, b) suggested that mosses produce ice nuclei that are very different from those produced by bacteria. Instead, they are proteins that show only distant relationship to the classical bacterial ice nuclei. Moffett *et al.* suggest that these ice nuclei are used as a water harvesting mechanism by the bryophytes, removing it from atmospheric moisture rather than from the bryophyte cells.

On the other hand, some bacteria do have a sneaky trick to gain entry into plant cells. These are a small number of ice-nucleating bacteria (Moffett 2015). The bacteria use certain proteins to induce freezing that damages the plants, permitting the bacteria to gain nutrients (Lindow 1983). But for bryophytes, it is possible that they help the plants gain water (Moffett 2015). Ice crystals are hygroscopic, gathering water from the atmosphere. This could be an advantage following the desiccating effects of freezing. Size matters, and smaller ice nucleating bacteria could prevent large crystal formation by out-competing the larger bacteria, a phenomenon used by Florida orange growers to prevent ice damage to the oranges on cold nights. Moffett found that all mosses and liverworts tested have active ice nucleation. This benefit for bryophytes survived as a water-gathering mechanism. Moffett suggested a number of hypotheses:

1. Ice nucleation is a ubiquitous feature of bryophytes.
2. Ice nucleation is used as a water-gathering mechanism.
3. Ice nucleation is of greater selective advantages to bryophytes growing in habitats such as rock and tree surfaces.
4. Ice nucleation in bryophytes is due to a surface expressed protein.
5. Ice nuclei from bryophytes become airborne and influence atmospheric processes.

All of these hypotheses need to be tested. Could the finding of a protein on the surface of the liverwort be a product of some unknown bacterium that didn't have the right conditions to appear in culture, rather than of the liverwort (see Kazda *et al.* 1980; Vesty *et al.* 2020)?

Weber (2016) provided evidence that spores (Figure 88) of *Polytrichum commune* (Figure 24) in the atmosphere are ice nucleators. This nucleation ability was active at -7°C when the spores were contaminated with bacteria, compared to -12°C for spores contaminated with microorganisms. Hence, moss spores can affect precipitation patterns, with the more common contaminated spores having the greater effect by causing freezing at a higher temperature.



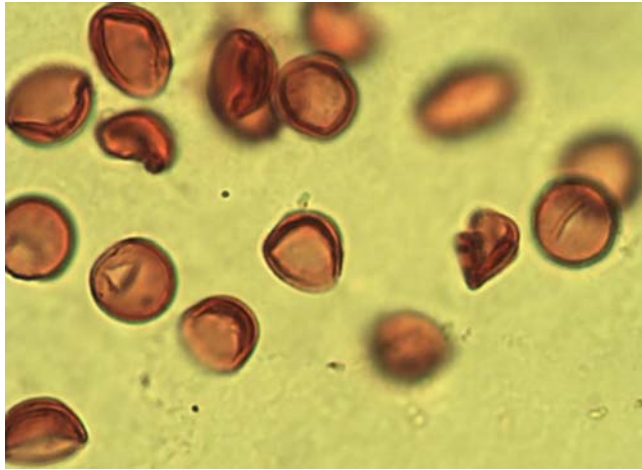


Figure 88. *Polytrichum commune* spores; these serve as ice nucleators in the atmosphere and are active at even higher temperatures when they have bacteria. Photo by Global Pollen Project, through Creative Commons.

## Nutrients

Šoltés *et al.* (2015) attempted to understand the seasonal variation of bryophytes in a calcareous mire in Slovakia. In a detailed examination of *Campylium stellatum* (Figure 89) and *Drepanocladus cossonii* (Figure 90), they found that distribution of these two mosses was limited primarily by decreasing concentrations of  $\text{NH}_4^+$  and increasing concentrations of  $\text{NO}_3^+$ . They determined that this seasonal variation in bryophyte cover was the result of the synergistic relationship with the nitrifying bacteria and by the unstable water table. The bacteria were instrumental in the decomposition of the organic substances in the soils, thus returning nutrients that benefitted the bryophytes.



Figure 89. *Campylium stellatum*, a species limited primarily by decreasing concentrations of  $\text{NH}_4^+$  and increasing concentrations of  $\text{NO}_3^+$ . Seasonal variation in this bryophyte cover resulted from a synergism with nitrifying bacteria and by an unstable water table. Photo from Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.



Figure 90. *Drepanocladus cossonii*, a species limited primarily by decreasing concentrations of  $\text{NH}_4^+$  and increasing concentrations of  $\text{NO}_3^+$ . Seasonal variation in this bryophyte cover resulted from synergism with nitrifying bacteria and by unstable water table. Photo by Hermann Schachner, through Creative Commons.

Some plants, especially graminoids, require silica (Si). It increases resistance to various forms of stress. But Si has limited availability to plants because of its insolubility. Bryophytes grow in locations where high levels of silica are present in rocks, so Hu *et al.* (2019) investigated the associated bacteria in the widespread moss *Hypnum plumaeforme* (Figure 91). They did indeed find that a strain in the bacterial genus *Kosakonia* was able to release Si from feldspar and quartz. These bacteria significantly increased the water-extractable Si in the soil, improved Si uptake by *Zea mays*, and promoted seedling growth. Hence the bryophyte rhizoids can provide the environment needed for the bacteria that release needed Si. We need research on this partnership role in habitats where both grasses and bryophytes grow. And do any of the bryophytes use silica?



Figure 91. *Hypnum plumaeforme*; the bacterium *Kosakonia* lives in the moisture provided by rhizoids of this moss and is able to release Si from feldspar and quartz. Photo by Janice Glime.

Epiphyllous liverworts can benefit nitrogen-fixing bacteria and *Cyanobacteria* by maintaining leaf moisture for a longer period of time, thus improving the usable N content in the canopy (Bentley & Carpenter 1980).



## Decomposition

When one thinks of bacteria in natural habitats, decomposition usually comes to mind. Nevertheless, we know that decomposition of bryophytes is notoriously slow (Fenton *et al.* 2010). Instead, the bryophytes retain high levels of soil carbon, retain excessively high soil water content, cool the soil, and slow nutrient cycles.

*Sphagnum* (Figure 12) decomposition can require specialized bacteria, with the abiotic environmental conditions having more importance than in other systems because of this bacterial specialization (Kulichevskaya *et al.* 2007). These bacteria are primarily members of the phyla **Actinomycetota**, **Planctomycetota**, and **Pseudomonadota** (Alphaproteobacteria). Kulichevskaya *et al.* found that the numbers of **Bacillota** and **Bacteroidota**, which are believed to be the primary decomposers in eutrophic wetlands, are low. As the decomposition reached its final stage, the numbers of **Planctomycetota** increased. Representatives of the **Pseudomonadota** were able to utilize galacturonic acid, the only low-molecular-weight organic compound detected in the water samples of the decomposing peat. The bacterial community involved in *Sphagnum* decomposition appears to be fundamentally different from that which decomposes the dead plant parts in eutrophic ecosystems at neutral pH. Even where *Sphagnum* is present, decomposition of the other bryophytes is significantly higher than that of the *Sphagnum* (Lang *et al.* (2009). The loss of mass in these other species correlates with the initial nitrogen, without influence of incubation conditions.

Kulichevskaya *et al.* (2010) named a new genus and species [*Bryobacter aggregatus* (Figure 39) in **Acidobacteriota**] for three strains of chemo-organotrophic bacteria isolated from acidic *Sphagnum* bogs (Figure 12). These bacteria preferred substrates of sugars (heteropolysaccharides, galacturonic acid, and glucuronic acids) – substances released during *Sphagnum* decomposition. These grew at pH 4.5-7.2 and 4-33°C.

Again in 2014 Kulichevskaya *et al.* described a new species, genus, and family of bacteria from *Sphagnum*. The species, *Roseiarcus fermentans*, is a microaerophilic fermentative bacterium in the **Pseudomonadota**.

Bamforth (2007) noted that protozoa are important in stimulating bacterial activity for decomposition. In a tropical forest in Puerto Rico, he found that the high moisture content of the tropical rainforest litter (including bryophytes) and soils provided the connected soil water needed for protozoan transport. Often there needs to be consideration of protozoan potential because of their dormancy status. Nevertheless, the large numbers of protozoa suggest that a major proportion of these contribute to stimulation of the bacterial decomposition for this organic matter.

Mikola and Hintikka (1956) experimented with decomposition of five forest litter types. One of these was the moss *Pleurozium schreberi* (Figure 92). Others were the grass *Deschampsia flexuosa* (Figure 93), shrub *Alnus incana* (Figure 94), deciduous tree *Populus tremula* (Figure 95), and conifer tree *Pinus sylvestris* (Figure 96). Of these, the *Pleurozium schreberi* litter had the lowest bacterial number and highest fungal count. The researchers noted that the related moss *Hylocomium splendens* (Figure 97) is very acid and decomposes differently from tracheophyte

leaves (Mikola 1954). Since these mosses are closely related species and occur in overlapping acidic habitats, it is possible that the acid conditions are unfavorable to bacteria while being favorable to the fungi.

Relative to lichen-dominated sites, bryophytes are associated with higher soil nutrient concentrations and a greater production of easily decomposable substrates that provide better maintenance of microbial activities (Ohtonen & Vare 1998). Do the bryophytes contribute to these better conditions, or are they simply indicators of the better conditions?



Figure 92. *Pleurozium schreberi*, a species, when compared with litter from four tracheophytes, had the lowest bacterial number and highest fungal count. Photo by Bob Klips, with permission.



Figure 93. *Deschampsia flexuosa*, a grass used by Mikola and Hintikka for comparison of bacterial activity in decomposition. Photo by James K. Lindsey, through Creative Commons.





Figure 94. *Alnus incana* leaf, a shrub used by Mikola and Hintikka for comparison of bacterial activity in decomposition. Photo by Vassil, through public domain.



Figure 95. *Populus tremula* leaf, a tree used by Mikola and Hintikka for comparison of bacterial activity in decomposition. Photo by Willow, through Creative Commons.



Figure 96. *Pinus sylvestris* litter, a conifer used by Mikola and Hintikka for comparison of bacterial activity in decomposition. Photo by Beentree, through Creative Commons.



Figure 97. *Hylocomium splendens*, a species that decomposes differently from tracheophyte litter, possibly due to its acidity. Photo by Claire Halpin, with permission.

Bastardo (1979) experimented with decomposition in *Fontinalis antipyretica* (Figure 98). Satake and Miyasaka (1984) found, by using TEM, that the leaves of the aquatic liverwort *Solenostoma vulcanicola* (Figure 99) exhibit rod-shaped bacteria and numerous holes in the liverwort cell walls. They suggested that these bacteria contribute to the decomposition of this liverwort.



Figure 98. *Fontinalis antipyretica* with silt and microorganisms. Photo copyright Malcolm Storey, with online permission.



Figure 99. *Solenostoma vulcanicola*, a leafy liverwort species that gets numerous holes in its cell walls due to rod-shaped bacteria. Photo courtesy of Angela Ares.



Van Tooren *et al.* (1988) found that nutrients released by decomposing bryophytes in spring and summer are incorporated by the tracheophytes, thus ensuring their retention in the system. In ecosystems where they are associated with nitrogen-fixing bacteria, such as mires and grasslands, they enhance the N in the ecosystem (Oechel & van Cleve 1986). Hence the nutrient content of the bryophytes affects the nutrient cycle of the whole ecosystem.

## Fauna and Bryophagy

Bacteria often play a role in feeding the animals that live among the bryophytes. In peatlands, *Sphagnum* is often a suitable substrate for a number of Protozoa (Mieczan 2006). The bacterivorous Protozoa were in the highest numbers in all the moss samples, whereas the algivorous ones were the lowest.

Nematodes are common among bryophytes in some habitats. Among these, members of the genus *Panagrolaimus* (Figure 100) are bacterial feeders that are known from terrestrial mosses in both the Antarctic and temperate ecosystems (Shannon *et al.* 2005). In a Balkan oak forest, Lazarova *et al.* (2000) found a similar relationship, with bacterial feeders being the most abundant group of nematodes on the moss *Hypnum cupressiforme* (Figure 101). Merrifield (1992) likewise found that the moss-dwelling nematode *Plectus* sp. (Figure 102) is a bacteria feeder.



Figure 100. *Panagrolaimus davidi*, in a nematode genus that has bacterial feeders that live among bryophytes. Photo from Smithsonian, through Creative Commons.

Many tardigrades are well adapted to living among mosses. They have the ability to dry out and rehydrate under the same water regimes as their moss hosts. Although one group of tardigrades has a stylet that permits them to feed on mosses, some of the tardigrades, particularly smaller ones, feed on bacteria that they find among the mosses (Tardigrada 2005; Schill *et al.* 2011).

Bryophyte communities often have associated arthropods. There is a body of evidence that many of these arthropods feed on the associated bacteria (Varga 1992).



Figure 101. *Hypnum cupressiforme*, where nematode residents are predominantly bacteria feeders. Photo by Kurt Stüber, through Creative Commons.



Figure 102. *Plectus murrayi*; a moss dweller in this genus is a bacteriovore. Photo from Bold Systems, by A. Velasco, through Creative Commons.

Although we often think of isopods as scavengers, they can be quite common among and under bryophytes. *Porcellio scaber* can come to the surface to feed on the softer apical tissues at night (Hribljan & Glime, in prep.). Because bryophytes have many substances that are difficult to digest, it is likely that they need some help. Zimmer (1999) found that oxidation of phenolics, common in many bryophytes, is primarily due to endosymbiotic bacteria. Furthermore, the gut has oxygen zones such that the outer, peripheral portion is anaerobic while the inner portion is aerobic. This range of conditions permits both aerobic and anaerobic bacteria to survive in the gut. Furthermore, the beginning of the gut is acidic, whereas the hindgut is neutral. It is not clear if these bacteria are gained from the bryophytes, but their presence could make bryophytes a good source of food.

Isopods also have bacteria in the gut that help them break down complex carbohydrates (Zimmer & Brune



2005). On the other hand, phenolics such as those present in mosses can reduce the gut flora needed for breaking down lignocelluloses (Zimmer 1999; Zimmer & Brune 2005). When the gut flora was reduced they were unable to hydrolyze gallotannins. When they ingested gallic acid, it reduced both the palatable fungi and the bacteria, but at the same time it increased the gut microflora. Thus, it would appear they cannot benefit from eating tracheophyte litter and bryophytes at the same time. This suggests that eating foods with hydrolyzable tannins, as found in some mosses, can inhibit the digestion of other foods in the diet of this species.

Pyszko *et al.* (2019) pointed out that we still lack an understanding of the gut bacterial flora of the moss-eating insects. Among the true bugs, the moss bugs (**Peloridiidae**; Figure 103) are obligately associated with endosymbiotic bacteria (Kuechler *et al.* 2013). The Malpighian tubules (part of the excretory system) have most of their nuclei infected by **Pseudomonadota** in the genus **Rickettsia** (Figure 104). The connection to bryophytes as food is not clear. Could the bugs subsequently eat the excreted uric acid complex? Or might the feces benefit from these bacteria, permitting the bugs to reingest them and benefit from them?



Figure 103. *Hemiodoecellus fidelis* (**Peloridiidae**) on *Sphagnum*, a moss bug that cultures *Rickettsia* bacteria in its Malpighian tubules. Photo by Simon Grove, through Creative Commons.

Using two bryophagous species of beetles in the **Byrrhidae** [*Simplocaria semistriata* (Figure 105) and *Curimopsis paleata* (Figure 106)], Pyszko *et al.* (2019) found that the gut flora differed considerably from the abdominal flora in the same individual beetle (Figure 107). Furthermore, both differed substantially from the substrate surface bacterial flora. The dominant bacteria in the guts and abdomens were all **Pseudomonadota**: *Novosphingobium* (Figure 28), *Bradyrhizobium* (Figure 1, Figure 53), *Ralstonia* (Figure 108), and *Caulobacter* (Figure 109). These bacteria are involved in detoxification of secondary metabolites or in nitrogen fixation. Since these genera are less common in the substrate surface samples, it is likely that they are associated with the specific ability of bryophages to feed on mosses.

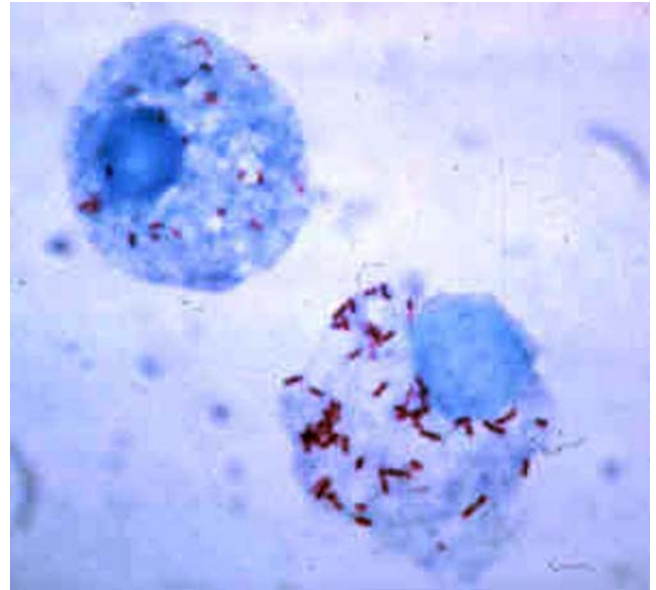


Figure 104. *Rickettsia rickettsii*, a bacterium found in the Malpighian tubules of the moss bugs (**Peloridiidae**). Photo from CDC, through Creative Commons.



Figure 105. *Simplocaria semistriata*, a bryophyte-eating beetle that seems to have a gut flora that helps it digest bryophytes. Photo by Boris Loboda, through Creative Commons.



Figure 106. *Curimopsis paleata*, a bryophyte-eating beetle that seems to have a gut flora that helps it digest bryophytes. Photo by M. Virtala, through Creative Commons.



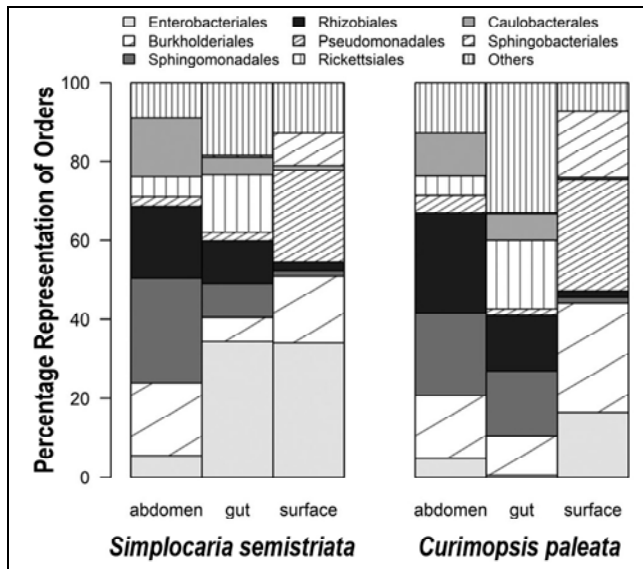


Figure 107. Composition of orders of bacteria in communities associated with abdomens, guts, and surfaces of the bryophyte-eating beetles *Simplocaria semistriata* and *Curimopsis paleata*. Modified from Pyszko *et al.* 2019.

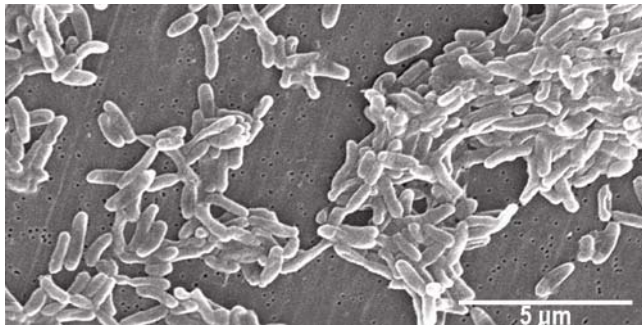


Figure 108. *Ralstonia mannitolilytica*, a bacterium involved in nitrogen fixation or detoxification of secondary metabolites and that occurs in the guts of the bryophyte beetles *Curimopsis paleata* and *Simplocaria semistriata*. Photo by Judith Noble-Wang, CDC, through public domain.



Figure 109. *Caulobacter crescentus*, a bacterium involved in detoxification of secondary metabolites or nitrogen fixation and that occurs in the guts of the bryophyte beetles *Curimopsis paleata* and *Simplocaria semistriata*. Photo from USDA, through public domain.

Some of the bacteria found in the **Byrrhidae** (pill beetles) guts are nitrogen fixers, *e.g.* *Bradyrhizobium* (Figure 1, Figure 53) (Pyszko *et al.* 2020). Since nitrogen is typically deficient in plants (Benemann 1973), the ability to extract more of it from food items may be especially beneficial. Rapid travel through the gut reduces this ability (Pyszko *et al.* 2020). Therefore, having nitrogen fixers in the guts of bryophyte eaters may be useful. Other benefits may include detoxification, such as the ability of the bacteria *Novosphingobium* (Figure 28) and *Ralstonia* (Figure 108) to degrade phenols and aromatics.

In bryophyte-dwelling *Cytilus sericeus* (Byrrhidae; Figure 110) treated with bactericides and fungicides, the bactericides actually had a positive effect on egg hatching and larval development, whereas the fungicides were detrimental to their fitness, particularly during hatching (Pyszko *et al.* 2020). When the larvae were supplied with adult feces, the feces did not improve fitness. Hence, the beneficial fungi are associated with the eggs, but are not transmitted in the feces. Could the bryophytes be providing bactericides that make the environment favorable to the developing eggs and larvae?



Figure 110. *Cytilus sericeus* on moss; bactericides actually had a positive effect on egg hatching and larval development. Bryophytes are likely to provide these bactericides in nature. Photo by James K. Lindsey, with permission.

Wolf and Rockett (1984) assessed the bacteria in the alimentary canals of two oribatid mites (*Rhysotritia* sp. (Figure 111) and *Pergalumna* sp.). These included *Acinetobacter* (Figure 112), *Actinomycetota*, *Alcaligenes* (Figure 113), *Bacillus* (Figure 9), *Citrobacter* (Figure 18), *Corynebacterium* (Figure 114), *Flavobacterium* (Figure 115), *Mycobacterium* (Figure 116), and *Pseudomonas* (Figure 10). The frequency of *Bacillus* and *Pseudomonas* was considerably lower in mites taken directly from natural habitats than from those found in moss-soil habitats. Both of these bacterial genera are common on mosses, so it is possible that the moss was the source of the bacteria. After being cultured in the lab (with no moss), both mite species showed dramatic shifts in their gut flora.





Figure 111. *Rhysotritia* sp., a mite that occurs on mosses and has a variety of bacterial genera in its gut. Photo by Scott Justis, with permission.

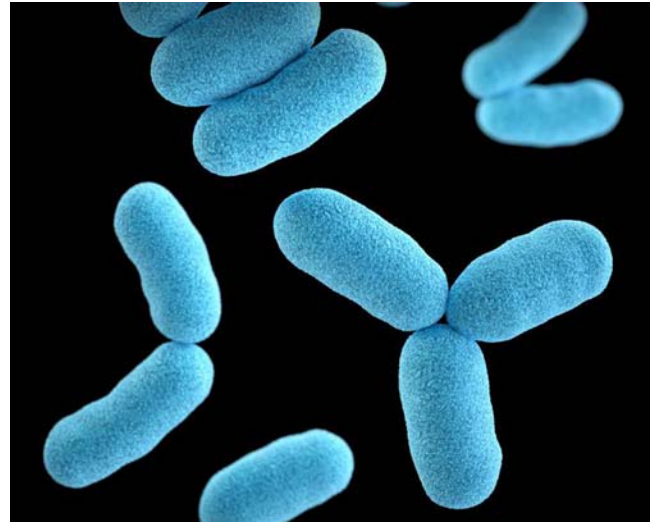


Figure 114. *Corynebacterium diphtheriae* SEM, in a genus that occurs in the alimentary canals of two oribatid mite genera. Photo by Jennifer Oosthuizen, CDC, through public domain.

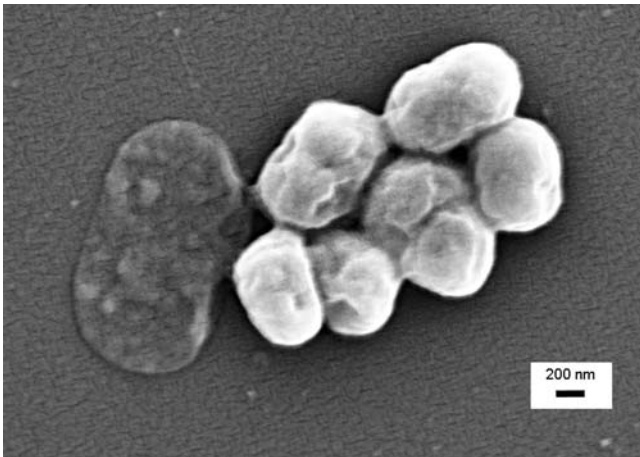


Figure 112. *Acinetobacter baumannii* SEM, in a genus that occurs in the alimentary canals of two oribatid mite genera. Photo by Vader1941, through Creative Commons.



Figure 115. *Flavobacterium columnaris*, in a genus that occurs in the alimentary canals of two oribatid mite genera, shown here in the gill of a chinook salmon. Photo from USFWS, through public domain.

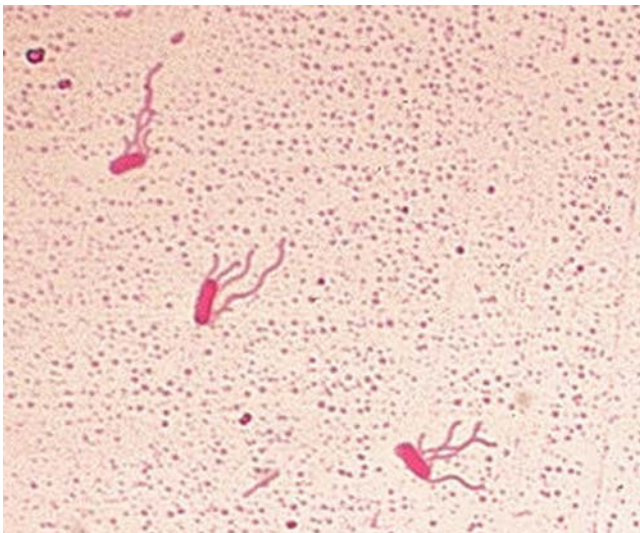


Figure 113. *Alcaligenes faecalis*, in a genus that occurs in the alimentary canals of two oribatid mite genera. Photo by W.A. Clark, CDC, through public domain.



Figure 116. *Mycobacterium tuberculosis* SEM, in a genus that occurs in the alimentary canals of two oribatid mite genera. Photo by NAIAD, through Creative Commons.



Moquin *et al.* (2012) found that for both **soil crusts** and mites the dominant phyla of bacteria were **Bacteroidota**, **Acidobacteriota**, and **Pseudomonadota**. The bacterial community and prevalence of **Bacteroidota** in the bryophytic crusts appear to be affected by high carbon availability. The bacterial communities associated with the bryophytic crusts are distinctly different from those of the cyanobacterial crusts and soils. **Acidobacteriota** prevailed in the mites, and the bacteria present in the gut are the same as those known as symbionts in *Tetraponera* (Figure 117) ants.



Figure 117. *Tetraponera punctulata*, an ant that has **Acidobacteria** as gut bacteria symbionts. Photo by Farhan Bokhari, through Creative Commons.

Mammals also may benefit from bacteria by getting more energy from bryophytes than would be possible otherwise. Pikas (*Ochotona princeps*, Figure 118) store plant foods for winter. They manipulate the decomposition of their food by storing with them plants with a high content of secondary compounds, including bryophytes (Dearing 1997). This permits them to store the plants for longer periods and to maintain higher levels of biomass and nutrients until they are eaten. Eating plants with high phenolic compounds is delayed until the phenolic content has decreased due to the microbial activity.



Figure 118. *Ochotona princeps*, an alpine rodent (pika) that stores plants with secondary compounds among its stored foods to preserve them longer. Photo by Linette Elliott, through Creative Commons.

Bjorkvoll *et al.* (2009) suggested that the Svalbard reindeer (*Rangifer tarandus platyrhynchus*, Figure 119) may be a specialist in consuming mosses. The fermentation chambers of the rumen are increased in size and have a very high number of fiber-digesting rumen bacteria. *Polytrichum* (Figure 24) was the most frequent moss consumed.



Figure 119. *Rangifer tarandus platyrhynchus* (Svalbard reindeer), apparently a specialist in moss consumption, probably due to the large number of bacteria in the rumen. Photo by Buibuione, through Creative Commons.

## Pathogens

Not all bacteria are friendly symbionts among their bryophyte neighbors. Lawton and Saidasan (2009) showed that *Physcomitrium patens* (Figure 67) is susceptible to a range of bacterial pathogens that can infect and multiply on the moss. In defense against these pathogens, it uses a variety of mechanisms: production of reactive oxygen species, synthesis of secondary metabolites, changes in gene expression, and activation of the programmed cell death pathway. These responses can be elicited by toxins as well as directly by the bacteria and are under genetic control.

The lab rat of mosses, *Physcomitrium patens* (Figure 67), is susceptible to a range of bacterial pathogens that can infect and multiply on the moss plants (Lawton & Saidasan 2009). One of the responses of the moss is to produce **reactive oxygen species**, as well as synthesis of secondary metabolites, changes in gene expression, and activation of the programmed cell death pathway.

One of the common bacteria on bryophytes is *Bacillus cereus* (Figure 9) (Sabovljević *et al.* 2010). On the other hand, the leafy liverwort *Lophocolea heterophylla* (Figure 120) and moss *Polytrichum commune* (Figure 24) produce antibiotics that are effective against this bacterium species (Nikolajeva *et al.* 2012) and in another study extracts of *Atrichum undulatum* were the most effective against *B. cereus* (Sabovljević *et al.* 2010).





Figure 120. *Lophocolea heterophylla*, a species that produces antibiotics against *Bacillus cereus*, a bacterial species that is antagonistic toward bryophytes. Photo by Kristian Peters, with permission.

In many of these studies, it is likely that more bacteria exist that are not stimulated to grow on the media being used. Kazda *et al.* (1980) cultured bacteria from 122 samples of *Sphagnum* (Figure 12) and other moss vegetation using foot pad inoculation. They found that of the 759 foot pads examined 20% had noncultivable acid-fast *Bacillus* (Figure 9). The frequency was significantly higher in the *Sphagnum cuspidatum* habitat (Figure 121).

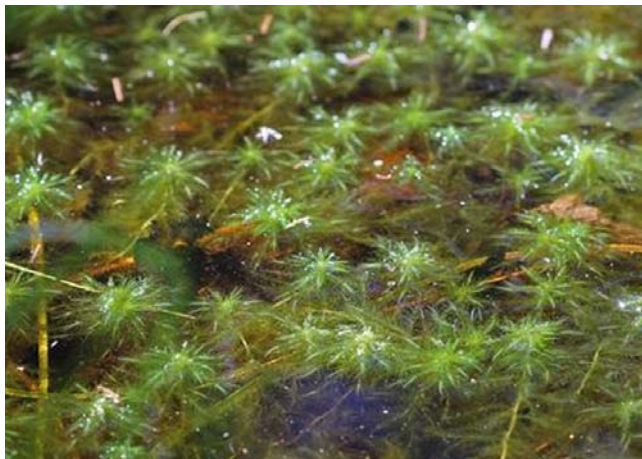


Figure 121. *Sphagnum cuspidatum*, a habitat where *Bacillus* has a high frequency. Photo by Rob Routledge, through Creative Commons.

This suggests that bryophytes could be reservoirs of bacteria that are pathogenic to other organisms. D'aoust *et al.* (1990) found that *Salmonella poona* (see Figure 16) and *S. arizonae* (see Figure 16) are frequently encountered in fertile eggs of pet turtles (*Trachemys scripta elegans*, Figure 122) and in the mosses used for packing the turtles. Since these species of bacteria became resistant to the antibiotics, the turtles were taken off the market to protect the children who would otherwise choose them as pets and possibly get infected by the bacteria.



Figure 122. *Trachemys scripta elegans*, a species of turtle that carries *Salmonella poona* and *S. arizonae* from the mosses used for packing material. Photo by Jf268, through Creative Commons.

### Bacterial Source of Antibiotics Useful to Bryophytes

*Pantoea agglomerans* (Figure 7) is known from bryophytes, in particular *Sphagnum fallax* (Figure 123- Figure 124) (Opelt *et al.* 2007). This bacterial species is an active producer of antibiotics that are effective against many plant pathogens among the bacteria and fungi (Dutkiewicz *et al.* 2016). This species of *Pantoea* does this by competition, releasing antibiotics, and induction of plant resistance. Bryophytes such as *Sphagnum fallax* can serve as a reservoir for the bacteria so that they become available to animals and annual plants and plant parts (Opelt *et al.* 2007). It is further useful, especially to rooted plants, by preventing the penetration of harmful industrial contaminants. But how does this latter feature affect bryophytes? Could it hold high concentrations near the soil surface where they may be harmful to bryophytes?



Figure 123. *Sphagnum fallax*, a species that can serve as a reservoir of bacteria needed by other plants and animals. Photo by Hugues Tinguy, with permission.



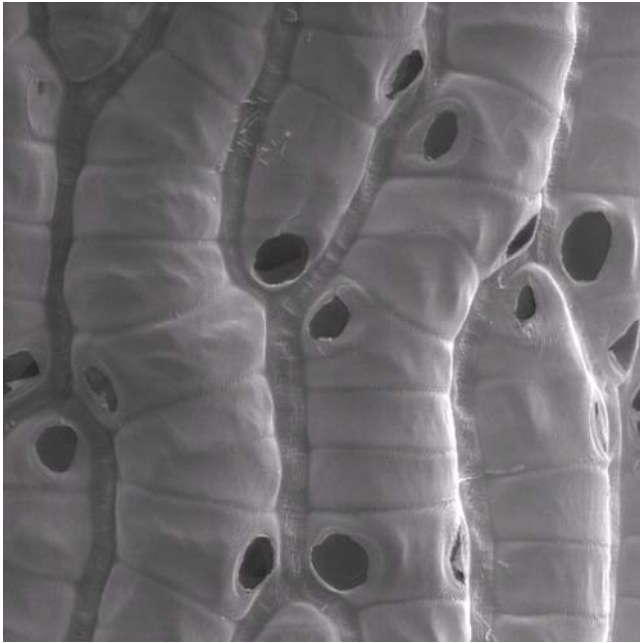


Figure 124. *Sphagnum fallax* hyaline cells with bacteria. Photo by Gabi Berg, with permission.

Opelt and Berg (2004) used *Syntrichia ruralis* (patches on sand dunes; Figure 85), *Aulacomnium palustre* (edge of non-calcareous mire; Figure 13), and *Sphagnum rubellum* (open part of mire; Figure 125) to represent typical moss species of nutrient-poor communities on the Baltic Sea coast of Germany and examine the antagonistic potential of bacteria associated with them. They found a high degree of specificity of the bacteria for the particular moss. This specificity was also manifest in the bacterial antagonistic behavior. For example, the antagonistic activity against the fungus *Verticillium dahliae* (Figure 126) ranged from 31% for *Sphagnum rubellum*, to 17% for *Aulacomnium palustre*, to 5% for *Syntrichia ruralis*. The antifungal role of the antagonistic bacteria is remarkable – 99% of those associated with mosses produced antifungal compounds.



Figure 125. *Sphagnum rubellum*, a strong antagonist against *Verticillium dahliae*. Photo by J. C. Schou, through Creative Commons.



Figure 126. *Verticillium dahliae* showing wilt disease. Photo by Howard F. Schwartz, through Creative Commons.

Out of the 52 species of bryophytes tested with 12 species of microorganisms, 29 (56%) were active against at least one of the test bacteria, but none exhibited any antifungal property (Banerjee & Sen 1979). Anyone who has tried to grow mosses in a closed space with a high humidity recognizes that fungi can be a threat to the moss health, so these antifungal roles of bacteria could be exceedingly important.

After searching through many papers on bacteria and bryophytes, it is unclear to me in many cases which bacteria can serve as pathogens to the bryophytes and which are either neutral or offer some antagonistic advantage to the bryophytes by inhibiting other bacteria or fungi. And some bryophytes produce antibiotics against specific antagonistic bacteria whereas others do not. This is a huge field of bryological interaction where we have just begun to scratch the surface in our understanding.

## Speculation

What a fantastic world of interaction! The bacteria got here first and developed all sorts of signals. Bryophytes took advantage of all those signals and developed quorum sensing. This made a close dependence possible and beneficial. So what might remain that we haven't even considered?

Could it be that the inhibition of gemmae germination on the thallus of *Marchantia* species (Figure 37, Figure 38) is due to a lack of germination signals from bacteria? The *Marchantia* produces secondary compounds that inhibit bacteria. Thus, germination might be prevented because the gemmae need hormones from the bacteria. On the other hand, *Methylobacterium marchantiae* (see Figure 6) isolated from *Marchantia polymorpha* (Figure 37) stimulates the surface expansion of isolated gemmae. But what is the timing? Under what environmental conditions? Does this only work if the thallus is dying? Do the numbers of bacteria signal the right season to germinate?

Spore germination signals are another potential role for bacteria. Some desert seeds have chemical inhibitors that prevent their germination. When there is a heavy rain, the inhibitors are washed away and the seeds germinate. This prevents them from germinating in a light shower or dew that provides insufficient water for continued survival of the germinated seedling. Do spores use bacteria as a similar signal? AHLs inhibit spore germination at high concentrations, but stimulate it at low concentrations.



Could the rain wash away the AHLs and permit the spores to germinate only when there is enough water for successful protonemal survival?

## Summary

The predominant members of the bryophyte bacterial communities belong to the **Pseudomonadota**. The **Bacillota** are common in some habitats, and a number of other bacterial phyla are less common.

I found it fascinating that the bryophytes have in many cases relied on bacteria to provide them with needed hormones for their growth and development. In such small plants, economy of resources is an important survival mechanism, so using products of reliably associated organisms is an adaptive advantage. These hormones were most likely available before the bryophytes originated, making the production of these substances by the bryophytes unnecessary.

A number of unrelated bryophytes require bacterially produced hormones to change stages in their life cycle. This is best known in producing buds on the protonema and in development of the buds into gametophores, explaining why some mosses won't develop in sterile culture with no added hormones.

The **Methanobacteria** typically are able both to break down methane to form CO<sub>2</sub> (then available to bryophytes for photosynthesis) and to fix atmospheric nitrogen (also used by bryophytes). This implies that these bacteria somehow provide anaerobic conditions within the cells to permit nitrogen fixation to occur.

At least some, perhaps all, bryophytes obtain vitamin B<sub>12</sub> from bacteria, but the physiological roll seems to be unknown – it does enhance growth and development in culture. The oxidative burst seen on rehydration of bryophytes can be a defense against pathogenic fungi and bacteria. Bacteria interfere with measurements of primary productivity of bryophytes, especially aquatic ones. They may help in the movement of water and nutrients up the bryophyte stems as water evaporates from the tips. Some bacteria provide freezing protection through ice nucleation. Others gain entry by causing freezing damage to bryophyte cell membranes. They can contribute to release of elements from rock, making them available to the community of plants. Bryophytes benefit bacteria by maintaining moisture for a longer period of time.

Some bacteria are pathogens to bryophytes, whereas others produce antibiotic compounds that protect the bryophytes from these pathogens. Bacteria are particularly important in producing antifungal compounds used by bryophytes, particularly liverworts. Bryophytes themselves produce many antibiotic compounds against bacteria. When the bacteria multiply, the bryophytes can respond to increased numbers (**quorum sensing**) to produce antibiotics needed for protection.

Many of the invertebrates that live among the bryophytes consume the bacteria or depend on them in other ways. Some bryophagous insects incorporate nitrogen-fixing bacteria in their gut to permit them to gain usable nitrogen from consumed bryophytes.

Bacteria associated with some bryophytes might prepare them for consumption by giving access to nutrients that were bound in recalcitrant tissues.

As a newly explored habitat, bryophytes have revealed new species and even new families of bacteria. Furthermore, many bacteria remain as unculturable, likely comprising a large number of new species. Their roles could be important to both the bryophytes and the larger plant community.

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# CHAPTER 19-2

## BRYOPHYTE BACTERIA EFFECTS ON COMMUNITIES

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# CHAPTER 19-2

## BRYOPHYTE BACTERIA EFFECTS ON COMMUNITIES



Figure 1. *Fontinalis dalecarlica* showing collection of sediments that harbor many bacteria, including *Methylocystis* that oxidizes  $\text{CH}_4$ , releasing  $\text{CO}_2$ . Photo by Jean Faubert, with permission.

### Community Effects

During and van Tooren (1990) noted that bryophytes occur in microhabitats that are formed by the physical environment and typically modified by the tracheophyte vegetation occurring with them, but the bryophytes are typically treated as if they are isolated from other plants and other organisms in the environment. More realistically, we are now beginning to realize the importance of the interactions between bryophytes and other organisms. These interactions are involved in competitive, parasitic, symbiotic, and mutualistic relationships. During and van Tooren pointed out that information regarding the relationships of bryophytes with other organisms, including bacteria, is essential for understanding mineral nutrition, carbon economy, herbivory, growth, development, and the overall ecological role of the bryophytes.

Reboledo and León (2021) again pointed out the importance of bryophyte-microorganism interactions. They pointed out that these interactions had developed during coevolution of the bryophytes with microorganisms. Some of the interactions took the place of substances the bryophytes would otherwise have needed to produce themselves, thus saving them energy. They also avoided complex pathways that responded to environmental differences and changes such as seasons.

Sun *et al.* (2017) used bryophyte removal experiments to learn that absence of bryophytes caused a change in the soil microbial community in the conifer-dominated forest and an ericaceous shrubland of the alpine Tibetan Plateau. Frahm *et al.* (2012) suggested that bacterial contamination may affect the antibiotic effect of bryophytes on seed germination.

Ma *et al.* (2017a) examined the bacterial communities on four moss species [*Campyliadelphus polygamus* (Figure 2), *Cratoneuron filicinum* (Figure 3), *Grimmia pilifera* (Figure 4), *Pylaisia polyantha* (Figure 5)]. They found a total of 279 genera comprised of 558 OTU's (operational taxonomic units). Of the 16 bacterial phyla found, the **Pseudomonadota** and **Actinomycetota** were the two most abundant phyla. The most common bacterial genera were *Bosea*, *Cellvibrio*, *Friedmanniella*, *Jatrophihabitans*, *Lapillicoccus*, and *Oligoflexus*. The two wet-habitat mosses (*Campyliadelphus polygamus*, *Cratoneuron filicinum*) had similar bacterial communities, differing from those of the two relatively dry habitat species (*Grimmia pilifera*, *Pylaisia polyantha*) that also had similar bacterial communities. The bacterial communities in the summer and autumn were most similar on each moss species. However the season was not the most important factor in causing community differences.





Figure 2. *Campyliadelphus polygamus*, a moss species that hosts mostly *Pseudomonadota* and *Actinomycetota*. Photo by Jean Faubert, with permission.



Figure 3. *Cratoneuron filicinum*, a moss species that hosts mostly *Pseudomonadota* and *Actinomycetota*. Photo by David T. Holyoak, with permission.



Figure 4. *Grimmia pilifera*, a moss species that hosts mostly *Pseudomonadota* and *Actinomycetota*. Photo by Wayne Lampa, through Creative Commons.

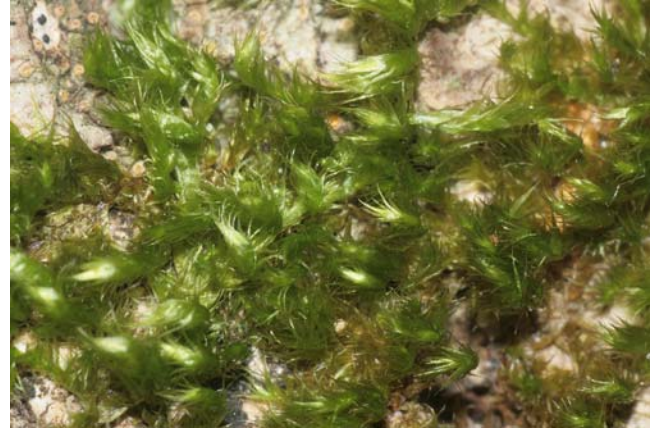


Figure 5. *Pylaisia polyantha*, a moss species that hosts mostly *Pseudomonadota* and *Actinomycetota*. Photo by Hermann Schachner, through Creative Commons.

Tang *et al.* (2016) were curious about the specificity of the bacteria on the bryophytes. They investigated ten liverworts and ten mosses from Tibet, China, using sequencing techniques. Six of the mosses had bacterial communities with a higher community similarity, but the remaining four mosses had communities that were more similar to those of the ten liverworts. Tang and coworkers concluded that the phylogeny of hosts has a strong influence on the associated bacterial community and that niche also plays important roles when the hosts are phylogenetically more similar.

Harris and Tibbles (1997) compared bacterial productivity in four Antarctic habitats. These included soils from four different habitats: beneath moss beds, from nests of snow petrel (*Pagodroma nivea*), exposed unvegetated soil (polygon soil) 5 m away from nests, and exposed polygon soil on nunataks without nests of breeding birds. The moisture levels from nest entrances and beneath mosses were much higher than in those from unvegetated exposed polygons. Mosses also modify temperatures, providing much cooler summer temperatures than exposed polygons on continental Antarctica, which are greater than 20°C at midday, and exhibited less temperature fluctuation. Harris and Tibbles considered these moss beds to be bacterial "hotspots," although based on temperature "coolspots" might be more appropriate. They considered that bacterial productivity in moss soils was typically nutrient limited, whereas in the polygons moisture was a more important factor.

Opelt and Berg (2004) considered the bryophytes to serve as a diverse community reservoir of bacteria that provided antibiotics against plant pathogens. Koua *et al.* (2015), in Japan, found that many of the bacteria associated with bryophytes played critical roles in soil nutrient enrichment, especially in nitrogen fixation. They seem to be especially important as hosts of nitrogen-fixing bacteria, contributing to biogeochemical cycling (Cornelissen *et al.* 2007).

Vollár *et al.* (2018) found that among the 42 bryophyte species in their study, the families **Brachytheciaceae** and **Amblystegiaceae** produced the greatest numbers of antiproliferative extracts – extracts that worked against the proliferation of cancer cells. *Plagiomnium cuspidatum* (Figure 6) seemed to be the most active, affecting 8 bacterial strains. As in several other bryophyte studies (*e.g.*



Bodade *et al.* 2008; Liu & Wang 2010; Liyanage *et al.* 2015; Sabovljević *et al.* 2010), *Staphylococcus aureus* (Figure 7) was the most susceptible to the antiproliferation activity. *Paraleucobryum longifolium* (Figure 8) exhibited the highest activity.



Figure 6. *Plagiomnium cuspidatum*, most active among 42 species of bryophytes, affecting 8 bacterial strains. Photo by Hermann Schachner, through Creative Commons.

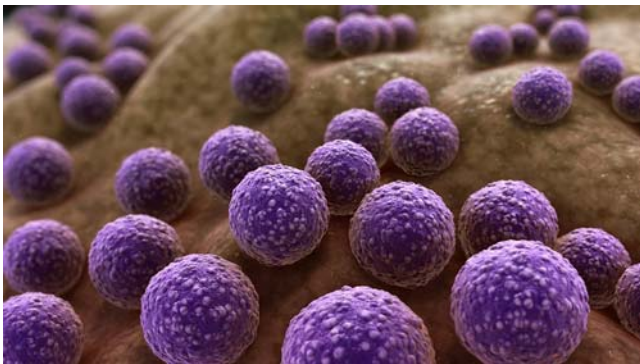


Figure 7. *Staphylococcus aureus*, a species that among the most susceptible to inhibition by bryophyte extracts. Photo from <www.scientificanimations.com>, through Creative Commons.



Figure 8. *Paraleucobryum longifolium*, the species with the greatest antibacterial activity among 42 species of bryophytes tested. Photo by Hermann Schachner, through Creative Commons.

Koua *et al.* (2015) reiterated that little is known about the bryophyte-associated microbial diversity or their role in

soil enrichment and nitrogen fixation. As community members, the bacteria associated with bryophytes have the potential to infect other members of the community. This is especially true for plant pathogens, but the bryophytes can also serve as a refuge for bacteria that affect animals and fungi.

Zhu *et al.* (2006) assayed 60 bryophyte species for their antibacterial activity and found that 93.3% of the species demonstrated antibacterial activity against at least two of the seven tested bacterial species [*Priestia megaterium* (Figure 9) – syn. = *Bacillus megaterium*, *Bacillus subtilis* (Figure 10), *Bacillus thuringiensis* (Figure 11), *Escherichia coli* (Figure 12), *Pseudomonas aeruginosa* (Figure 13), *Pseudomonas putida* (see Figure 13), and *Staphylococcus aureus* (Figure 7)]. This activity was particularly strong in the liverwort genera *Conocephalum* (Figure 14), *Frullania* (Figure 15), *Herbertus* (Figure 16), *Marchantia* (Figure 17), *Mastigophora* (Figure 18), and *Porella* (Figure 19). But what does this activity against human tracheophyte pathogens mean for the communities where these bryophytes live?

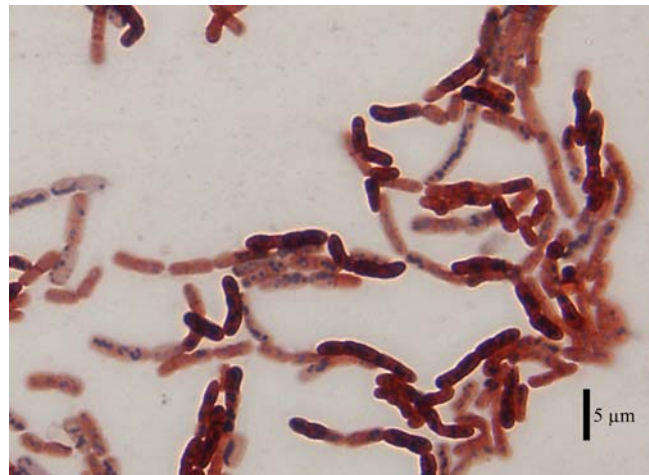


Figure 9. *Priestia megaterium* DSM-90 cells colored with Sudan black and safranin, a species that is affected by antibacterial compounds from bryophytes in China. Photo by Osmoregulator at English Wikipedia, through Creative Commons.

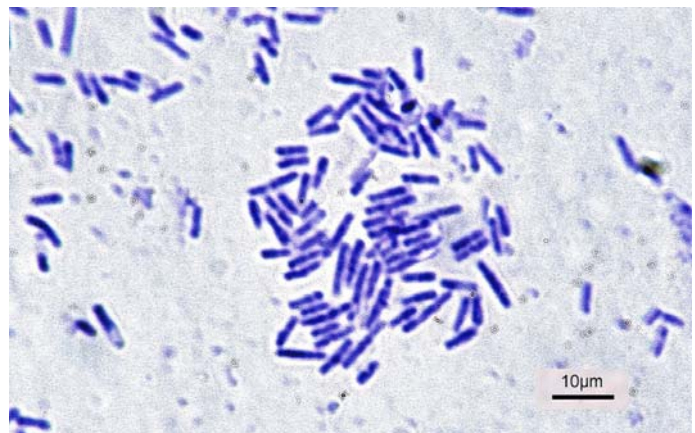


Figure 10. *Bacillus subtilis*, a species that is affected by antibacterial compounds from bryophytes in China. Photo by Graham Beards, through Creative Commons.





Figure 11. *Bacillus thuringiensis*, a species that is affected by antibacterial compounds from bryophytes in China. Photo by Dr. Sahay, through Creative Commons.

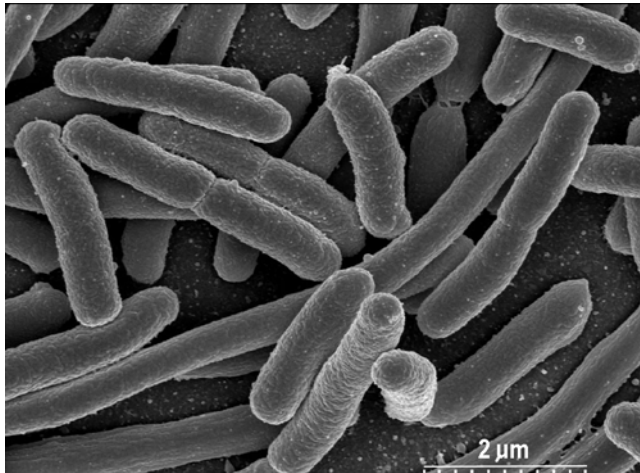


Figure 12. *Escherichia coli*, a species that is affected by antibacterial compounds from bryophytes in China. Photo by Rocky Mountain Laboratories, NIAID, NIH - NIAID, through public domain.

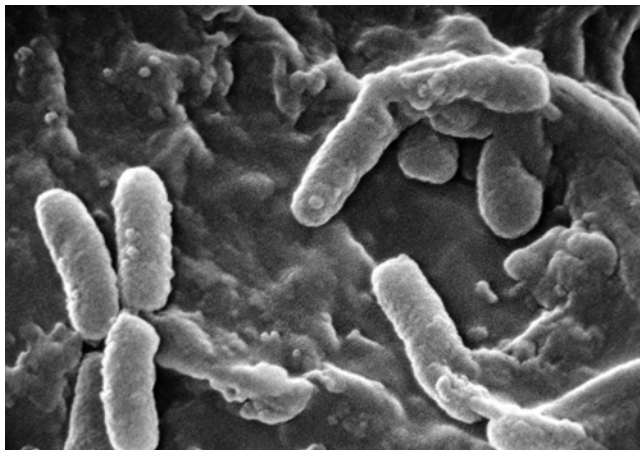


Figure 13. *Pseudomonas aeruginosa* SEM, a species that is affected by antibacterial compounds from bryophytes in China. Photo by Janice Haney Carr, CDC, through public domain.



Figure 14. *Conocephalum*, a genus in China with particularly strong antibacterial activity. Photo by Don Loarie, through Creative Commons.



Figure 15. *Frullania dilatata*, in a genus in China with particularly strong antibacterial activity. Photo by Bernd Haynold, through Creative Commons.



Figure 16. *Herbertus aduncus*, in a genus in China with particularly strong antibacterial activity. Photo from Botany Website, UBC, with permission.





Figure 17. *Marchantia polymorpha*, in a genus in China with particularly strong antibacterial activity. Photo by Denis Barthel, through Creative Commons.



Figure 18. *Mastigophora woodsii*, in a genus in China with particularly strong antibacterial activity. Photo by Jo Denyer, with permission.



Figure 19. *Porella platyphylla*, in a genus in China with particularly strong antibacterial activity. Photo by Hermann Schachner, through Creative Commons.

In one study *Pseudomonas putida* (see Figure 13) is most sensitive to the extracts of mosses, and *Bacillus subtilis* (Figure 10) is most sensitive to the extracts of liverworts (Zhu *et al.* 2006). *Staphylococcus aureus* (Figure 7) is the most resistant of the seven tested bacteria to the extracts of both liverworts and mosses, contrasting

with its widespread susceptibility to bryophyte extracts in a number of other studies (*e.g.* Bodade *et al.* 2008; Liu & Wang 2010; Liyanage *et al.* 2015; Sabovljević *et al.* 2010). This suggests that some bryophytes could serve as a refuge for the *Staphylococcus aureus*, but there is evidence that suggests that the antibiotics against *S. aureus* from some of the bryophytes are only produced when the bacteria are present (Sabovljević *et al.* 2010).

The importance of this inducible response to the community has not been investigated. If the antibiotics manufactured by bryophytes are effective against these bacteria that are not known to be pathogenic to bryophytes, how might these antibiotics affect other bacteria in the ecosystem? What selection pressures kept these antibiotic properties in the library of secondary compounds produced by bryophytes that weren't harmed by them? How can we explain that *Bacillus subtilis* is the most sensitive of the seven tested bacteria to liverwort extracts and that *Pseudomonas putida* is the most sensitive to moss extracts (Zhu *et al.* 2006)? And why do 99% of the bacteria found with mosses produce antifungal compounds (Opelt and Berg 2004)? Why are there no compounds produced by any liverwort as protection against fungi (Banerjee & Sen 1979)? Why should bryophytes produce so many compounds that protect other organisms, but at the same time depend on bacteria to produce some compounds that protect the bryophytes?

Many of the bacteria associated with bryophytes appear to be unknown because they do not grow on standard media (see Vesty *et al.* 2020).

Are some of these undetected bacteria the sources of any of the antibiotic compounds that we attribute to the bryophytes? Are we missing some antibiotic compounds when we culture the bryophytes axenically before testing them, thus failing to elicit any inducible responses (see Sabovljević *et al.* 2010)? Among these bacteria, how important are they to soil nutrient cycling? Are they nucleators that affect our weather? Are the bryophytes a reservoir for ice-nucleators that may be beneficial or detrimental to other kinds of plants? What sorts of selection pressures exist for these less known or unknown bacteria?

Using DNA and RNA techniques we are able to assess such differences without the need to name the bacteria involved. In China, Wang *et al.* (2018) found abundant bacteria associated with all the mosses they sampled and identified in this manner. These were mostly in the phyla *Pseudomonadota* and *Actinomycetota*. Their OTU level hierarchy separated the bacteria into two main branches of aquatic vs terrestrial. The aquatic habitat showed larger differences in the bacterial community composition than did the terrestrial habitat. Thus, the habitat of the host bryophyte is an important factor in determining the community.

## Streams and Rivers

Bryophytes in streams typically are covered with bacteria, making it difficult to assess productivity of the bryophytes alone (Arscott *et al.* 1998). These are difficult to remove, and the role of the bacteria in producing CO<sub>2</sub> that bryophytes can use in photosynthesis needs to be assessed. Furthermore, the bryophytes trap silt and organic



matter (Figure 20) that flows in the stream or river, making them an important habitat for some kinds of bacteria.



Figure 20. *Fontinalis antipyretica* with detritus that can serve as food for bacteria and macroinvertebrate scrapers. Photo by J. C. Schou, with permission.

In their study of carbon sources used by primary consumers in two oligotrophic rivers, McWilliams-Hughes *et al.* (2009) found that tracheophyte macrophytes and **Cyanobacteria** were unimportant as food sources for insect scrapers. Rather, 98% of the scrapers exhibited  $\delta^{13}\text{C}$  values that were enriched with bryophyte  $\delta^{13}\text{C}$  values, especially when slow-flowing habitats were excluded from the analysis. *Fontinalis* sp. (Figure 21) was abundant in headwater (low order) streams, where it exhibited more dependence by scrapers than the dependence by scrapers associated with the *Drepanocladus* (Figure 22) sp. of the high-order streams (with higher order streams having more combining tributaries). This is consistent with the greater cover by bryophytes in the headwater streams. They suggested that scrapers might switch to marginal food sources such as bryophytes in the headwaters where productivity and nutrients are low. But what is really providing their food? Might it be the bacteria and other periphyton that are always associated with the mosses? I have seen a Dipteran larva eat "dirty" mosses (Figure 20) and watched the feces come out as clean moss fragments.



Figure 21. *Fontinalis antipyretica*, home for macroinvertebrate scrapers in streams. Photo by Claire Halpin, with permission.



Figure 22. *Drepanocladus aduncus*, in a genus in slower water in streams than that of *Fontinalis* and where scrapers are less dependent on it for food. Photo by Hermann Schachner, through Creative Commons.

Špoljar *et al.* (2012) found that when the bryophyte coverage was scattered the diversity of algae, protozoa, and meiofauna was governed by the amount of suspended organic matter and epiphytes. They concluded that this was the result of enrichment by seston travelling downstream. One can assume that bacteria are associated with this seston (Bowden *et al.* 2017), but what is their role? Are the bacteria food themselves, or are they only important in releasing nutrients from the seston?

Heino *et al.* (2015) examined the metacommunity structure by surveying the diatoms, bacteria, bryophytes, and invertebrates in three drainage basins in Finland. The species were mostly distributed independently of one another in the southernmost drainage basin, but in the northernmost drainage basin there were discrete community types. These relationships seem only to be related to geography and not to environmental heterogeneity. They suggested that environmental variables may vary between organismal groups.

Stream conditions would seem to be ideal for many kinds of bacteria. The constant supply of sediments that get carried during times of rapid flow are trapped by the bryophytes, where these sediments can accumulate. But what protections do the stream bryophytes have against this potential associated bacterial onslaught? Basile *et al.* (1998) used an acetone extract to assess the bacterial activity of the stream moss *Platyhypnidium riparioides* (Figure 23) against 11 strains of bacteria. They found that this extract was active against some Gram-negative strains. Are these antibacterial properties effective against the bacteria that normally inhabit this moss? Most of the testing has been with human pathogens, but early assessments did not determine the natural bacterial flora of the mosses.

What quickly becomes evident by these studies is that we know little about the bryophyte bacteria and their role in the stream ecosystems. Meyer *et al.* (2007) commented that whereas we have an understanding of stream fungal diversity and know that fungi are critical to the organic matter dynamics and food webs in headwater streams, we know little about bacterial diversity.





Figure 23. *Platyhypnidium riparioides*, a stream moss with antibacterial activity against 11 strains. Photo by J. C. Schou, with permission.

Demars *et al.* (2020) showed that addition of carbon caused a rapid increase in both photosynthesis and heterotrophic respiration in a stream. In the control stream, with no added carbon, the carbon exchange between the autotrophs and bacteria accounted for ~49% of the bacterial production and 37% of the net primary production during periods of stable flow. The researchers considered the bryophyte contribution to primary productivity to be negligible, so they did not include them in their food flow calculations. Furthermore, the added sucrose (carbon) in the experiment did not end up in the bryophytes. It is likely that such carbon exchange between the bacteria and bryophytes in an important occurrence when the predominant autotrophs are bryophytes.

Yakubik *et al.* (2000) noted that some bacteria in bryophyte mats contribute to denitrification. This can be accentuated in lower reaches of a stream where the water is slower. This lower flow rate results in less mixing and permits the bacteria to colonize the mosses more easily, and provides a longer residence time for the denitrification to occur.

On the other hand, Leppänen (2013) demonstrated that  $N_2$  fixation can occur in association with *Fontinalis dalecarlica* (Figure 1), although the rates are low. The highest activity was in the upper, green portion, compared to the lower, brown portion of the plants. In boreal forest streams in Finland, it oxidized  $CH_4$  at the highest rate among the boreal mosses investigated, which included peatland and feather mosses. It seems certain that the  $N_2$  fixation is dependent on an external energy source and is closely connected with the oxidation of  $CH_4$ . But  $N$  fixation must take place in an oxygen-free environment. As much as 74% of this fixation is tied to the *nifH* sequences best identified as the bacterium *Methylocystis* (Figure 24). This evidence, coupled with the high  $CH_4$  rates, suggests that *Fontinalis dalecarlica* is important in the  $CH_4$  of boreal rivers. The bacteria are located on the leaf, in the cavity between the leaf and stem, but can also occur inside the outer stem cells. There is a mucous-like substance in the cells of the moss that may be important in the relationship. Solheim *et al.* (2004) suggested that the mucous might result from or contribute to a symbiotic relationship between the bacteria and moss. On the other hand, Postgate (1998) suggested that it could be a protective strategy to create an anaerobic environment for the nitrogenase to work.

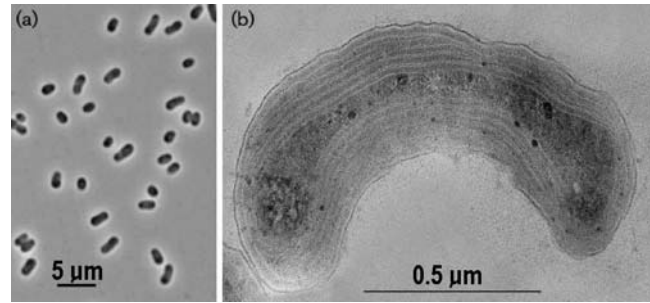


Figure 24. *Methylocystis bryophila* extracted from peat. **a.** Phase-contrast micrograph of cells. **b.** Electron micrograph of an ultrathin section of a cell. From Belova *et al.* 2013, with permission from Svetlana Dedys.

Martinez-Abaigar *et al.* (1993) evaluated the effects of organic pollution on the mosses *Fontinalis antipyretica* (Figure 20, Figure 21) and *Brachythecium rivulare* (Figure 25) and the leafy liverwort *Jungermannia exsertifolia* subsp. *cordifolia* (Figure 26) in rivers in Spain. They suggested that the morphology of the bryophytes influenced the sensitivity to the pollution, resulting from differences in their ability to capture suspended organic materials (Figure 20). This organic matter promotes the growth of bacteria and their ability to degrade and cause senescence in the bryophytes.



Figure 25. *Brachythecium rivulare*, a species that captures organic materials that promote the growth of bacteria. Photo by Hugues Tinguy, with permission.



Figure 26. *Jungermannia exsertifolia* subsp. *cordifolia*, a species that captures organic materials that promote the growth of bacteria. Photo by Claire Halpin, with permission.



The leafy liverwort *Solenostoma vulcanicola* (Figure 27) exhibits rod-shaped bacteria associated with numerous holes in its cell walls, suggesting that bacteria play a role in its decomposition (Satake & Miyasaka 1984).



Figure 27. *Solenostoma vulcanicola* in Japan, a species with colonies of rod-shaped bacteria that create numerous holes in its cell walls. Photo by Masaki Shimamura, with permission.

### Faunal Connections

Bryophytes can play a key role in food for stream nematodes (Dražina *et al.* 2014). Dražina *et al.* (2013) found that it is the suction-feeding nematodes that dominate among the stream bryophytes, the same group that is common among terrestrial mosses in Europe (Barbuto & Zullini 2006). The nematodes use their stylets to pierce the bryophyte cells and suck out the contents (Traunspurger 2002; Dražina *et al.* 2013). Other nematodes feed on the epiphyton and deposits associated with the bryophytes (Suren 1992; Majdi *et al.* 2011). Thus, not only do some stylet-feeding nematodes eat bryophytes, but bryophytes provide the substrate for detrital pathways for nematode food. Furthermore, uptake of bacterial-respired CO<sub>2</sub> by the bryophytes (and algae) finds its way into these photosynthetic bryophytes, thus increasing the food available to both stylet bryophyte-feeding nematodes and other invertebrates (Demars *et al.* 2021).

In contrast to many earlier studies, Demars *et al.* (2021) suggested that the aquatic bryophytes covered by periphyton might contribute to the macroinvertebrate diet, a suggestion already supported by some earlier researchers (Jones 1949; Dangles 2002; Parker & Huryn 2006; Carroll *et al.* 2016). This also contrasts with their earlier conclusion (Demars *et al.* 2020) that this bryophyte-periphyton association does not contribute much to primary productivity. Stream ecologists are beginning to rethink the role of bryophytes in streams.

### Antarctic

Christie (1987) contrasted the nitrogen in a wet carpet and dry peat in the Antarctic. The numbers of sulphate-reducing bacteria [*Desulfovibrio* (Figure 28) and *Desulfotomaculum* (Figure 29) and of *Clostridium* (Figure 30) were very low, even in the wet carpet. The low acetylene reduction activity of these bacteria and absence of *Azotobacter* (Figure 31) suggest that nitrogen fixation was not an important contributor to nitrogen present. There

furthermore were no nitrifying bacteria. However, there were substantial numbers of proteolytic and nitrate-respiring bacteria as well as a small number of denitrifying bacteria. The heterotrophic groups were more abundant in the wet carpet than in the dry turf.

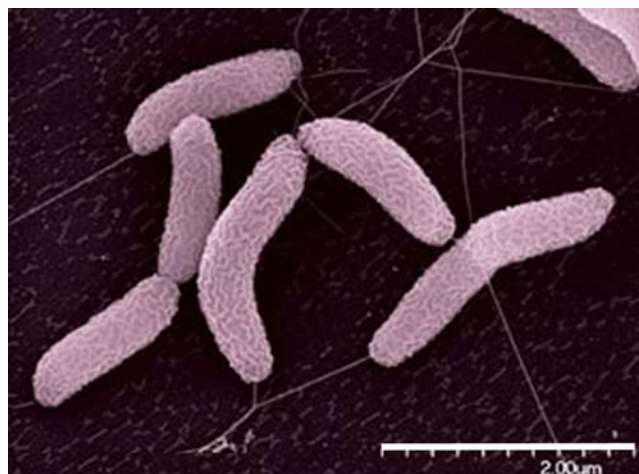


Figure 28. *Desulfovibrio desulfuricans*, a sulfate-reducing bacterium in a genus with low numbers in the Antarctic peat. Photo through public domain.



Figure 29. *Desulfotomaculum*, a sulfate-reducing bacterium with low numbers in the Antarctic peat. Photo by Manfred Rohde, through Creative Commons.

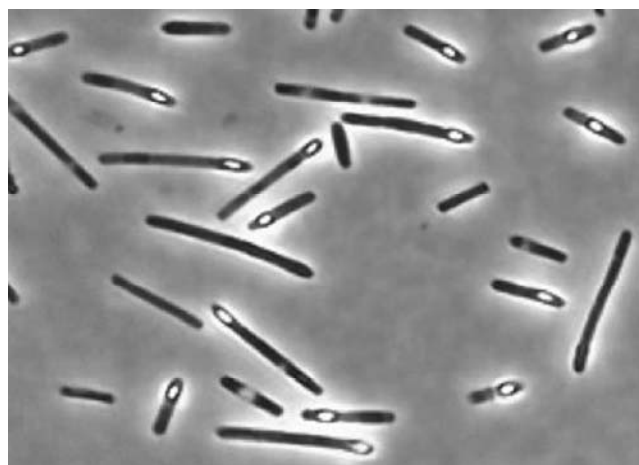


Figure 30. *Clostridium perfringens* sporulating, in a bacterial genus with low numbers in the Antarctic peat. Photo by Oregon State University, through Creative Commons.



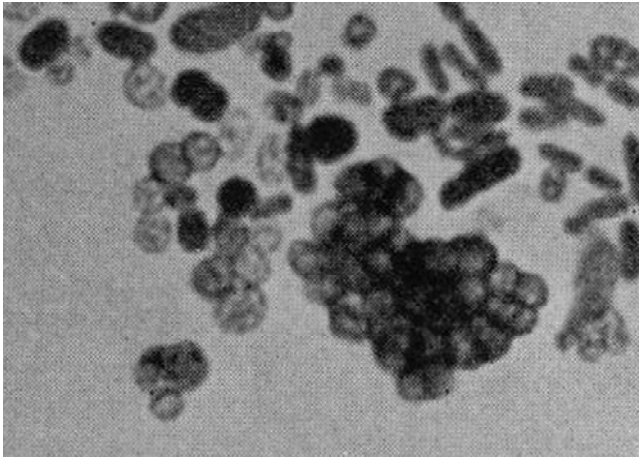


Figure 31. *Azotobacter*, a nitrogen-fixing bacterium that is absent in the Antarctic peat. Photo by Dan H. Jones, through public domain.

Park *et al.* (2013) investigated the neglected bacterial relationships among Antarctic bryophytes, using the moss *Sanionia uncinata* (Figure 32). They found that the *Pseudomonadota* was the dominant phylum, comprising 65.5% of the associated bacteria. The *Bacteroidota* (29.1%) and *Actinomycetota* (11.7%) were also important. The bacteria on the mosses exhibited zonation, with the *Alphaproteobacteria* comprising only 2% of the bacterial flora in the upper green parts, but were in significantly greater proportion at 22.2% in the lower brown portions. Park and coworkers considered it likely that there were specific relationships between these endophytes and the host moss.



Figure 32. *Sanionia uncinata*, a species with *Proteobacteria* comprising 65.5% of the associated bacteria in an Antarctic study. Photo by Hermann Schachner, through Creative Commons.

Raymond (2016) answered a question I raised earlier, before reading this – Is it the bryophyte or the bacterium that produces the external ice-binding proteins? A strong ice-pitting activity was known in the Antarctic moss *Bryum argenteum* (Figure 33). Raymond realized that this was a sign of ice-binding proteins that protect against freezing damage. He found that this ice-binding activity is the result of ice-binding proteins produced by bacteria living on the moss leaves. These were mostly

*Actinomycetota* and *Bacteroidota*. He found 42 bacterial ice-binding proteins. These operate in a symbiotic relationship with the moss, with the latter providing the sustaining energy source and the bacteria protecting the moss from freezing damage.



Figure 33. *Bryum argenteum*, an Antarctic moss with a strong ice-pitting activity. Photo by Claire Halpin, with permission.

### Arctic Alpine

Löffler *et al.* (2008) found that an increase in the occurrence of bryophytes and shrubs along an arctic-alpine gradient in Scandinavia would also increase the microbial activity. They also found that higher temperatures promoted greater soil microbial activity in the summer. Whereas these temperatures are expected to promote shrub growth as the climate warms, the higher moisture expected with climate change is expected to promote bryophyte growth. Both temperature and moisture increases should increase the microbial activity in the soil in heathlands in Scandinavia. Spruce forests (Figure 34) had the highest bacterial  $\alpha$ -diversity, whereas the aspen forests (Figure 35) exhibited greater turnover ( $\beta$ -diversity) and higher  $\gamma$ -diversity.



Figure 34. *Picea mariana* in Alaskan taiga, a forest type that had the highest bacterial  $\alpha$ -diversity in Scandinavia. Photo from NOAA, through public domain.





Figure 35. *Populus tremuloides* (aspen), a forest type that had the highest turnover ( $\beta$ -diversity) and higher  $\gamma$ -diversity of microbes in Scandinavia. Photo by Famartin, through Creative Commons.

### Boreal Forest

The boreal forest bryophyte bacterial communities have thus far been widely neglected. Only the **Cyanobacteria** (Figure 36) have attracted the attention of researchers. They will be covered in a separate chapter, but their relative role needs to be clarified here.



Figure 36. *Chroococcus*, one of the common **Cyanobacteria** associated with *Sphagnum*. Photo by M. Lorenz, through Creative Commons.

In boreal forests in Finland and elsewhere, the feather mosses *Hylocomium splendens* (Figure 37) and *Pleurozium schreberi* (Figure 38) predominate (Leppänen 2013). The N fixation activity associated with these mosses is well known (Meeks 1998; DeLuca *et al.* 2002; Gundale *et al.* 2012). As in *Fontinalis dalecarlica* (Figure 1), Gavazov *et al.* (2010) found higher nitrogen fixation in the upper portions of *Hylocomium splendens*. This suggests that light has an important role in the fixation (Meeks 1998; Gundale *et al.* 2012), implicating **Cyanobacteria**. But in the boreal forests of Finland, there was no significant amount of methane ( $\text{CH}_4$ ) oxidation activity, a phenomenon that Leppänen (2013) attributed to

the low concentrations of  $\text{CH}_4$  in the forest habitats and moisture conditions that are unsuitable for methanotrophs (Larmola *et al.* 2010). Since methane oxidation is often accompanied by nitrogen fixation, this leaves us wondering which organisms are contributing to these higher levels of nitrogen fixation in younger portions of the mosses.



Figure 37. *Hylocomium splendens*, a boreal forest feather moss that is important in hosting bacteria that fix nitrogen. Photo by Clare Halpin, with permission.



Figure 38. *Pleurozium schreberi*, a moss shown to be less important in driving bacterial communities in the boreal forest than the forest type. Photo by Hugues Tinguay, with permission.

DeLuca *et al.* (2002) reported that the **Cyanobacteria** (Figure 36) association with *Pleurozium schreberi* (Figure 38) alone fixes  $1.5\text{--}2.0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  in the mid to late successional forests of northern Scandinavia and Finland. Gavazov *et al.* (2010) found that liverworts did not fix detectable amounts of  $\text{N}_2$  in the boreal/sub-Arctic forests. Lichens had the highest rates of fixation, but because of their greater biomass, the mosses served as the nitrogen sink through their nitrogen fixation associations.

Rodríguez-Rodríguez *et al.* (2022) hypothesized that moss species would be the driver in the composition of the ecologically important bacterial communities associated with them. They quantified changes in bacterial communities as a function of host species [mosses *Pleurozium schreberi* (Figure 38) and *Ptilium crista-castrensis* (Figure 39)] and forest type [coniferous black spruce (Figure 34) versus deciduous broadleaf trembling



aspen (Figure 35)] in eastern Canada. But in fact, it was forest type, not moss host species, that was the main factor affecting bacterial community composition on the mosses.



Figure 39. *Ptilium crista-castrensis*, a moss shown to be less important in driving bacterial communities in the boreal forest than the forest type. Photo by M. Porcius Cato, through Creative Commons.

Among these boreal bryophytes, at least some of the nitrogen fixation seems to occur without the activity of **Cyanobacteria** (Figure 36), as for example associations with members of *Dicranum* (Gundale *et al.* 2011). *Pleurozium schreberi* (Figure 38) and *Sanionia uncinata* (Figure 32) exhibited relatively similar mean rates of  $N_2$ -fixation ( $46.1 \mu g N g^{-1} day^{-1}$  and  $52.4 \mu g N g^{-1} day^{-1}$  respectively), despite a dominance of **Alphaproteobacteria**  $N_2$ -fixers 21.4% vs 6.8% **Cyanobacteria**) on *Pleurozium schreberi* and a dominance of **Cyanobacteria** (79%) on *Sanionia uncinata*, with only 7.2% **Alphaproteobacteria** as  $N_2$ -fixers (Holland-Moritz *et al.* 2018).

Holland-Moritz *et al.* (2018) indicated that the moss microbiome in the boreal forest is highly diverse, includes many undescribed taxa (including an undescribed phylum), and is a major contributor to nitrogen fixation beyond that from the **Cyanobacteria**. The average richness is 924 phylotypes per sample, dominated by 8 bacterial phyla: **Pseudomonadota** (44.8% of reads across all samples), **Acidomycetota** (10.8%), **Verrucomicrobiota** (9.8%), **Bacteroidota** (9.3%), **Cyanobacteria** (6.5%), **Candidate phyla WPS-2** (5.7%), **Planctomycetota** (5.2%), and **Actinomycetota** (4.2%). Cutler *et al.* (2017) found similar relative abundances on *Pleurozium schreberi* (Figure 38). Many of these bacteria are novel and undescribed lineages within the **Alphaproteobacteria** sub-phylum and the **Verrucomicrobiota** phylum (Bragina *et al.* 2015).

But many of the boreal forest phylotypes could not be classified beyond phylum or class (Holland-Moritz *et al.* 2018), emphasizing how little we know about these communities. Contrasting with the study of Rodríguez-Rodríguez *et al.* (2022) in eastern Canada, the identity of the moss species was more important than the site in

determining the composition of the bacterial communities associated with the mosses near Fairbanks, Alaska, USA, with moss species accounting for 63% of the variation in bacterial community composition. The pleurocarpous moss *Sanionia uncinata* (Figure 32) had communities quite distinct from those of the other 6 moss species [*Pleurozium schreberi* (Figure 38), *Tomentypnum nitens* (Figure 40), *Aulacomnium palustre* (Figure 41), *A. turgidum* (Figure 42), *Dicranum elongatum* (Figure 43), and *Sphagnum capillifolium* (Figure 44)]. Of the bacteria on *Sanionia uncinata*, 59% were unique to that moss species. Only 77% of the 30 most abundant phylotypes present on the other mosses were present on *S. uncinata*, whereas 90% of the 30 most abundant phylotypes were found on all the other moss species.



Figure 40. *Tomentypnum nitens*, a species of fen mosses with bacteria that are similar to most of the other dominant fen mosses near Fairbanks, Alaska, USA. Photo by Hermann Schachner, through Creative Commons.



Figure 41. *Aulacomnium palustre*, a species of fen mosses with bacteria associates that are similar to those of most of the other dominant fen mosses near Fairbanks, Alaska, USA. Photo by Kristian Peters, through Creative Commons.





Figure 42. *Aulacomnium turgidum*, a species of fen mosses with bacteria that are similar to those of most of the other dominant fen mosses near Fairbanks, Alaska, USA. Photo by Mark Hill, with permission.



Figure 43. *Dicranum elongatum*, a species of fen mosses with bacteria that are similar to those of most of the other dominant fen mosses near Fairbanks, Alaska, USA. Photo by Hermann Schachner, through Creative Commons.



Figure 44. *Sphagnum capillifolium*, a species of fen mosses with bacteria that are similar to those of most of the other dominant fen mosses near Fairbanks, Alaska, USA. Photo by Blanka Aguero, with permission.

The moss-associated bacteria are likely to be anoxygenic phototrophs capable of carbon fixation via Rubisco, with an ability to utilize by-products of photorespiration from hosts via a glyoxylate shunt (Holland-Moritz *et al.* 2018). Furthermore, whereas most of the mosses were dominated by **Acetobacteraceae** (**Alphaproteobacteria**), **Acidobacteriaceae** (**Acidobacteriota**), and **Methylophilales** (**Verrucomicrobiota**), *Sanionia uncinata* (Figure 32) had either a low abundance or total absence of these bacterial taxa. It was dominated instead by **Comamonadaceae** (**Betaproteobacteria**), **Nostocaceae** (**Cyanobacteria**), and **Chitinophagaceae** (**Bacteroidota**). But why are these communities on *Sanionia uncinata* so different? Is it differences in microhabitat, or are the chemical defenders of these mosses different?

In an attempt to predict the effect of climate change on nitrogen fixation in the boreal forest, Gundale *et al.* (2012) assessed the effects of elevated temperatures on the relationship between bacteria and the feather moss substrate. They surmised that *Pleurozium schreberi* (Figure 38) may become a larger source of N in boreal forests relative to *Hylocomium splendens* (Figure 37) as climate warming progresses. Although the feather mosses have been considered to be cyanobacterial hosts, it is quite possible that these are also accompanied by Methanobacteria or other forms of non-cyanobacterial bacteria that are able to fix atmospheric nitrogen.

De Long *et al.* (2016) found that global warming altered the microbial communities in the soil, favoring communities based on bacteria. These, however, were mediated by mosses and shrubs, varying with successional stage. Mosses can serve as a buffer, like a heavy quilt, in modifying the soil temperature, as well as slowing the loss of moisture from the soil.

### Peatland Bacterial Flora

Peatlands (Figure 45), often dominated by *Sphagnum* (Figure 46) as the keystone species, contain dead plant material accumulated over thousands of years (Kamal & Varma 2008). This habitat covers ~5-8% of the world's surface and contains ~3-3.5 times the amount of carbon stored by tropical rainforests. Weston *et al.* (2015) noted the importance of *Sphagnum* as a keystone species in a habitat that holds more than one-third of the terrestrial carbon on the planet Earth.



Figure 45. Peatland in Estonia. Photo by Martin Küttim, through Creative Commons.





Figure 46. *Sphagnum* spp. in Estonia. Photo by Martin Küttim, through Creative Commons.

In this role, *Sphagnum* (Figure 46) plays an important role, along with its associated microbiome, in carbon and nitrogen cycling (Kamal & Varma 2008). The combination of high acidity, low temperatures, and extremely low concentrations of nutrients in many *Sphagnum* habitats suggests that we should find unique communities of bacteria associated with these bryophytes.

Kostka *et al.* (2016) noted that *Sphagnum* (Figure 46) displays a diverse community of microorganisms on its surface and within the tissues. The prokaryotes and fungi can act as mutualists, symbionts, or antagonists. In these roles, methanotrophic and nitrogen-fixing bacteria may provide up to 20-30% of *Sphagnum* carbon and nitrogen.

### Methane Oxidation

The production of methane (CH<sub>4</sub>) in peatlands has become a hot topic recently because of its potential role in global warming. Wetlands, including peatlands, are the largest natural sources of atmospheric methane (Raghoebarsing *et al.* 2005). Nevertheless, most of the methane formed in wetlands stays in wetlands, where it is recycled. *Sphagnum* (Figure 46) species in peatlands use this methane through symbiosis with partly endophytic methanotrophic bacteria. These bacteria occur both on the stem leaves and in the hyaline cells of the *Sphagnum*. The methane is rapidly oxidized by the bacteria to form CO<sub>2</sub> that is then fixed by the *Sphagnum* in photosynthesis and ultimately into plant sterols. The methane, through this pathway, provides 10-15% of the carbon source for *Sphagnum*.

Nevertheless, peatlands are a major source of methane (Larmola *et al.* 2010). But *Sphagnum*-dominated peatlands (Figure 45) have lower methane emissions than those known for other mire types. These researchers found that all 23 species of *Sphagnum* (e.g. Figure 46) in a boreal mire supported methanotrophic bacteria. Furthermore, transplanted *Sphagnum* with no indication of these methanotrophic bacteria exhibited the bacteria in their new

location, showing rapid movement and/or activation of the bacteria among the *Sphagnum*, and the importance of habitat. Water level is important in regulating the methane oxidation. The methanotroph *Methylocystis* (Figure 24) was present with *Sphagnum* plants that exhibited both active and inactive in methane oxidation, suggesting that its activity depended on water availability. Larmola and coworkers concluded that there is a loose symbiosis between the *Sphagnum* species and methanotrophic bacteria that contributes to 10-30% of the *Sphagnum* carbon.

In 2013, Belova *et al.* discovered a new species of *Methylocystis*, *M. bryophila* (Figure 24), that could convert methane to CO<sub>2</sub> and could also fix nitrogen. This species was isolated from an acidic *Sphagnum* (Figure 46) peat-bog lake in Germany and from a peat bog in northern Russia. They attributed the nitrogen-fixing capability to an aerotolerant nitrogenase.

The most abundant methanotrophs in peatlands typically are **Alphaproteobacteria** and **Gammaproteobacteria** (Kip *et al.* 2010). Within the hyaline cells, *Sphagnum* hosts other bacteria that decrease the O<sub>2</sub> concentration and increase the CO<sub>2</sub> concentration in these cells (Granhall & Hofsten 1976), thus favoring photosynthesis in the nearby photosynthetic cells and benefitting the **Cyanobacteria** living in the hyaline cells. These **Cyanobacteria** fix N<sub>2</sub> that could be used by both the moss and the other bacteria (Leppänen 2013). But *Sphagnum* (Figure 46) can also house methanotrophic bacteria in these same cells, and these bacteria are capable of N<sub>2</sub> fixation as well. These methanotrophic bacteria can provide up to 30% of the carbon in the moss (Raghoebarsing *et al.* 2005) because they can fix nitrogen and oxidize CH<sub>4</sub> at the same time (Leppänen 2013). Most of the N<sub>2</sub> is fixed in the dark in aerobic conditions in the peat, indicating activity of heterotrophic bacteria (Kravchenko & Doroshenko 2003).

### Nitrogen Sources

Aldous (2002) demonstrated that atmospheric nitrogen deposited among *Sphagnum* (Figure 46) plants was translocated to the capitula, with 11% to >80% in the lower and higher influx sites, respectively. Gerdol *et al.* (2006) noted that *Sphagnum papillosum* (Figure 47-Figure 48) was able to grow more when it had higher capitulum N concentrations. Both research groups noted that high water level favored the movement. Gerdol *et al.* (2006) found that the microbial nitrogen fixers were able to increase the nitrogen in the *Sphagnum* peatland. As with atmospheric nitrogen sources, these bacteria (no **Cyanobacteria** found) that occurred in senescing plant tissues, followed by upward transport of the fixed nitrogen, positively affected the growth of *Sphagnum papillosum*. In dry growing seasons, enhancement of fixed nitrogen in the rhizosphere promoted growth of tracheophytes that subsequently increased their competition against the *Sphagnum*. Experimental additions of nitrogen benefitted the *Sphagnum* only when the growing conditions inhibited the bacterial colonies.





Figure 47. *Sphagnum papillosum* in a peatland; microbial nitrogen fixers in senescing cells of this species are able to increase the nitrogen and benefit growth. Photo from Botany Website, UBC, with permission.



Figure 48. *Sphagnum papillosum*, a species positively affected by increased nitrogen that is often contributed by bacteria. Photo by David T. Holyoak, with permission.

Bragina *et al.* (2013) found that genes for nitrogenase were both high in abundance and diverse among the *Sphagnum* (Figure 46). These were usually specific for each *Sphagnum*. The methanotrophs, on the other hand, exhibited highly similar patterns among species. The sporophyte of the *Sphagnum* had a high proportion of specific **diazotrophs** (organisms able to grow without external sources of fixed nitrogen) (4%) but lacked methanotrophs. The nitrogen-fixing bacteria were highly specific and were transferred with the sporophyte.

Waughman and Bellamy (1980) found that nitrogen fixation rates were lower at the nutrient extremes, with that in bogs being especially low. The highest activity measured was  $>100 \text{ nmol C}_2\text{H}_4 \text{ mL peat}^{-1} \text{ d}^{-1}$ . Nitrogenase activity had a positive correlation with pH and K and a negative correlation with Ca. Lower latitudes have a greater nitrogenase activity in peat than do higher latitudes, even when temperatures are factored in. In the south German mires the nitrogen fixed annually by the heterotrophic bacteria were  $2100 \text{ mg N m}^{-2}$  in fens,  $530$  in poor fens, and  $70$  in bogs.

## Comparisons of *Sphagnum* Species

Bragina *et al.* (2012) compared the communities of bacteria on *Sphagnum fallax* (Figure 49) and *S. angustifolium* (Figure 50), two closely related species with distinct habitat nutrient preferences in weakly acid, mesotrophic situations influenced by minerotrophic groundwater. The two species also produce similar secondary metabolites. The two species exhibited high similarity (minimum of 95.5%) of bacterial colonization patterns. The interior of hyaline cells of the leaves were colonized primarily by **Alphaproteobacteria** (in **Pseudomonadota**). This group of bacteria was represented by a high degree of diversity, including *Acidocella*, *Acidisphaera*, *Phenylobacterium*, and *Rhodopila* as the major bacterial taxa on both species of *Sphagnum*, with no statistically significant differences between the microbial communities of the two *Sphagnum* species. The composition of the subdominant **Caulobacteraceae** did, however, vary between mosses, being more abundant with *S. fallax*. In this group *Phenylobacterium* was detected all over, whereas *Caulobacter* (Figure 51), *Gluconacetobacter* (Figure 52), *Methylocystis* (Figure 24), *Methylosinus*, and *Rhizobium* (Figure 53) occurred only with *S. fallax*. The associated microbial community fulfills functions that can only be accomplished by the cooperation with the *Sphagnum* community. These include nitrogen fixation, solubilizing phosphorus, and providing carbon from peat-derived methane (Raghoebarsing *et al.* 2005; Opelt *et al.* 2007c). Bragina and coworkers considered the moss-bacterial communities to have the same close relationships that are present between *Sphagnum* and the ecology and function of the bog ecosystem.



Figure 49. *Sphagnum fallax*, a species of weakly acid mesotrophic habitats with mostly **Verrucomicrobiota** and **Planctomycetota** as bacterial associates. Photo by Hugues Tinguy, with permission.





Figure 50. *Sphagnum angustifolium*, a species that grows in weakly acid, mesotrophic situations influenced by minerotrophic groundwater. This species is colonized by a highly diverse group of **Alphaproteobacteria** in the hyaline cells of the leaves. Photo by Hermann Schachner, through Creative Commons.



Figure 51. *Caulobacter crescentus*, in a genus that occurs with *Sphagnum fallax*, but not with *S. angustifolium*. Photo by USDA, through public domain.



Figure 52. *Gluconacetobacter* cellulose biofilm, a genus that occurs with *Sphagnum fallax*, but not with *S. angustifolium*. Photo through Creative Commons.

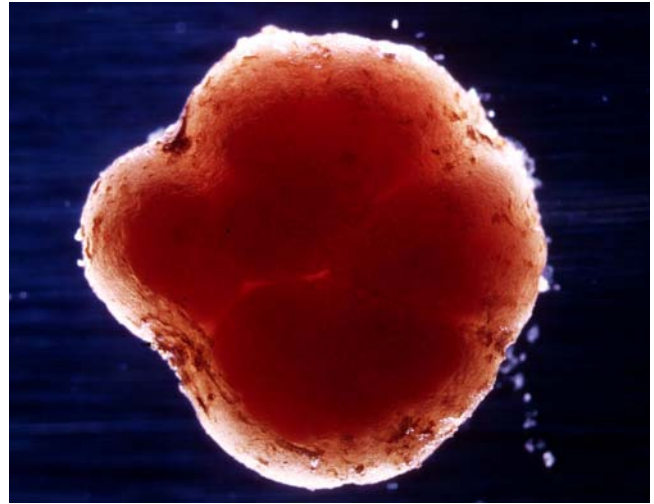


Figure 53. *Rhizobium* bacteria nodule, a genus that occurs with *Sphagnum fallax*, but not with *S. angustifolium*. Photo from CSIRO, through Creative Commons.

Bragina *et al.* (2012) also compared the bacterial diversity on *Sphagnum divinum* or *S. medium* (previously considered part of *S. magellanicum*) (Figure 54) and *S. fallax* (Figure 49) in three alpine bogs in Austria. *Sphagnum divinum/medium* characterizes strongly acid habitats with poor nutrients, whereas *S. fallax* inhabits weakly acid mesotrophic habitats. *Sphagnum divinum/medium* was inhabited mainly by **Gammaproteobacteria** (in Phylum **Pseudomonadota**), whereas associates of *S. fallax* (Figure 55) were mainly in the phyla **Verrucomicrobiota** and **Planctomycetota**. Although the bacterial colonies occurred in high abundance in the dead hyaline cells, they were always connected with living photosynthetic cells (Figure 55). Bragina and coworkers found that nutrient richness and pH were the most important determining factors for bacterial communities. They found it interesting that the bacterial diversity was transferred from the sporophyte to the gametophyte, contrasting with the transfer from the soil in tracheophyte communities. Microbial fingerprints showed that bacterial species from different bogs had a high similarity within the same bryophyte species. They considered the plant to plant transfer of bacteria, rather than through a soil intermediary, to be a possible explanation of the high specificity of *Sphagnum*-associated bacteria over long distances.



Figure 54. *Sphagnum cf. divinum*, a species of strongly acid habitats with poor nutrients, and mostly with **Gammaproteobacteria**. Photo by Michael L  th, with permission.



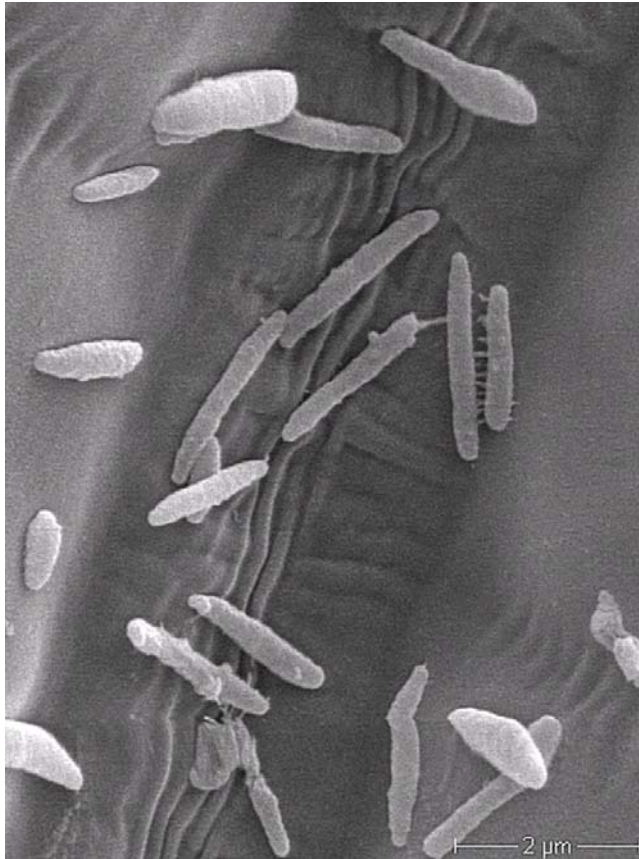


Figure 55. *Sphagnum fallax* bacteria SEM. Photo courtesy of Gabi Berg.

Shcherbakov *et al.* (2015) isolated bacterial populations from gametophytes of *Sphagnum* (Figure 54) from various geographic regions of Russia. Among the more than 400 strains isolated, ribosomal data indicated that the isolates were in the genera *Pseudomonas* (Figure 13) (20-57%), *Collimonas* (7-10%), *Flavobacterium* (Figure 56) (6-8%), *Burkholderia* (Figure 57) (5-6%), and *Serratia* (Figure 58) (3%). These are similar to the bacteria taxa reported for *Sphagnum* from the Austrian Alps (Bragina *et al.* 2015).



Figure 56. *Flavobacterium columnare* on gills of *Delistes luxatus*; this bacterial genus is common on Russian *Sphagnum*. Photo by S. Vanderkoo, through Creative Commons.

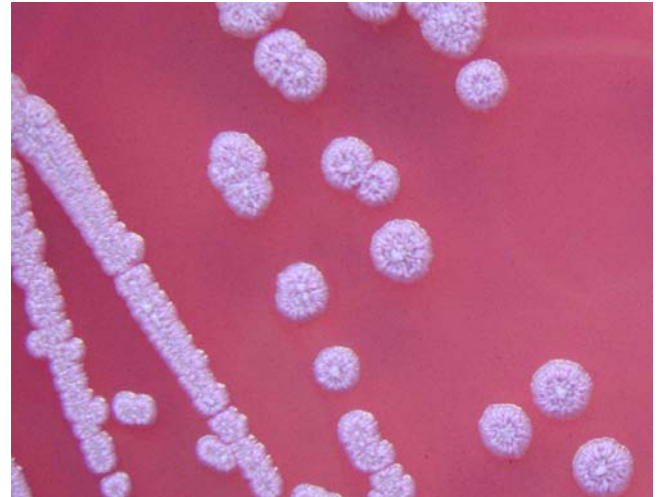


Figure 57. *Burkholderia pseudomallei*, in a genus of bacteria that are antagonistic toward bryophytes in Germany. Photo by Gavin Koh, through Creative Commons.



Figure 58. *Serratia marcescens* antibiogram, in a genus among the most common antagonists toward bryophytes in Germany. Photo by Stefan Walkowski, through Creative Commons.

Tian *et al.* (2019) compared the microbial community associated with the brown (lower) and green portions of *Sphagnum palustre* (Figure 59) peat. Alphaproteobacteria (in phylum Pseudomonadota) were dominant in all samples. Members of the phylum Acidobacteriota were abundant in the *S. palustre* peat, whereas Gammaproteobacteria (in phylum Pseudomonadota) dominated the brown layer. Cyanobacteria dominated the green portion. They considered the structural differences in the microbiome to be mainly due to microhabitat differences. The microbial communities of the *Sphagnum palustre* peat was significantly influenced by the water table and the total nitrogen content.





Figure 59. *Sphagnum palustre*, a species where **Alphaproteobacteria** is very frequent; **Gammaproteobacteria** dominates in the lower brown layer. Photo by Hugues Tinguy, with permission.

Opelt *et al.* (2007a, b) explored the bacterial relationships with *Sphagnum divinum/medium* (Figure 54) and *S. fallax* (Figure 49) from three locations in Germany and three in Norway. They particularly looked for bacteria that exhibited antagonistic activity against fungal pathogens; these comprised 48% of the isolates. *Sphagnum divinum/medium* housed 24% of the antagonists compared to only 19% for *S. fallax*. Nevertheless, *S. fallax* bacterial communities exhibited a significantly higher diversity  $\{H' = -\sum_{i=1}^s [(n_i/N)\ln(n_i/N)]\}$  than did the *S. divinum/medium* associates. More of the inhabitants of *S. divinum/medium* were specific to that species. The researchers suggested that the higher nutrient levels in the *S. fallax* habitat could explain the higher bacterial diversity.

Most of the antagonists in the Germany/Norway study were in the genera *Serratia* (Figure 58) (15%), *Burkholderia* (Figure 57) (13.5%), *Staphylococcus* (Figure 7) (13.5%), and *Pseudomonas* (Figure 13) (10%) (Opelt *et al.* 2007a). Whereas most of the antagonist strains had a high moss specificity, *Burkholderia* and *Serratia* had similar molecular fingerprints on both *Sphagnum* (Figure 49, Figure 54) species. A high proportion of the antagonist strains [*Hafnia* (Figure 60), *Pantoea* (Figure 61), *Staphylococcus*, and *Yersinia* (Figure 62)] are known as facultative pathogens of humans.



Figure 60. *Hafnia alvei*, in a genus of bacteria that is antagonistic toward bryophytes in Germany and also a facultative pathogen of humans. Photo by Antoine2003, through Creative Commons.

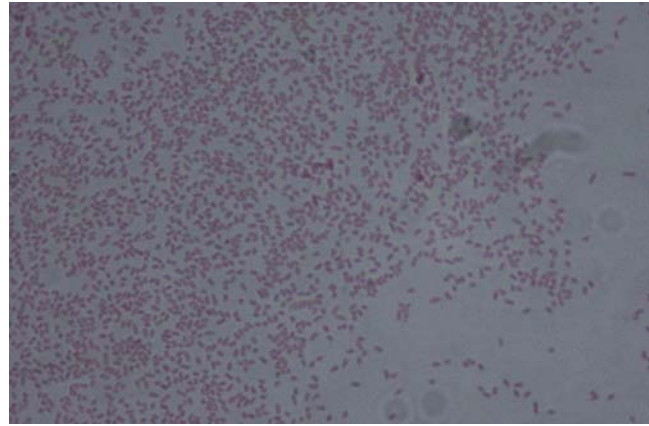


Figure 61. *Pantoea agglomerans* Gram stain, in a genus of bacteria that is antagonistic toward bryophytes in Germany and also a facultative pathogen of humans. Photo by Dr. Sahay, through Creative Commons.

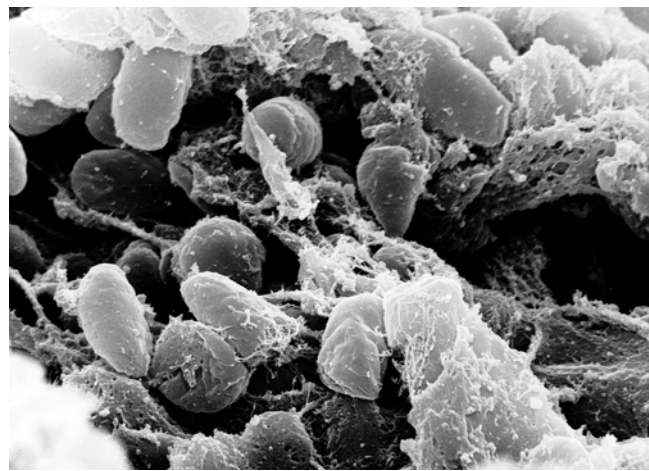


Figure 62. *Yersinia pestis* in gut of flea, in a genus among the most common antagonists toward bryophytes in Germany. Photo from CDC, through Creative Commons.

### Antibiotic Role

Bacteria associated with bryophytes often serve as protectors, providing the antibacterial or antifungal compounds needed to protect the bryophytes. Whereas Opelt *et al.* (2007c) found a high proportion (26%) of antifungal bacteria, they found only 0.4% antibacterial strains. Among these antagonists, there was a high diversity of *Burkholderia* (Figure 57) isolates in the **endophytic** (living within cell) and **ectophytic** (living on plant surface) habitats of *Sphagnum* (Figure 49, Figure 54). The researchers suggested that these antagonistic bacteria could account for the high level of antimicrobial activity of *Sphagnum*. It seems likely that the high level of antimicrobial compounds in at least some *Sphagnum* species could account for the specificity of the bacteria that grow there. The researchers also found a high diversity of nitrogen-fixing bacteria, especially inside *Sphagnum*.

Because of these antagonistic properties, Sarolta *et al.* (2010) sought new types of medically useful antagonists in the Borsáros Raised Bog in Romania. They explored the communication forms that make the bacterial antagonism successful, analyzing the biofilm formation of single strains and co-cultures.



Szentes *et al.* (2011) found that rhizobacteria produced plant-growth-promoting substances that interacted in various ways, including competing with pathogens for nutrients. This research team found *Bacillus* (Figure 10-Figure 11), *Cedecea*, *Delftia* (Figure 63), *Lysinibacillus* (Figure 64), *Pseudomonas* (Figure 13), *Serratia* (Figure 58), *Stenotrophomonas* (Figure 65), and *Viridibacillus*. *Bacillus*, *Pseudomonas*, and *Serratia* were the dominant bacterial genera associated with bryophytes. *In vitro* cultures showed that a high number of the isolates inhibited the growth of fungal and bacterial plant pathogens such as *Pectobacterium carotovorum* (Figure 66) or produced secondary metabolic substances with biocontrol properties. *Serratia fonticola* BB17 (see Figure 58) and *Pseudomonas fluorescens* (Figure 67) BE8 were the most efficient against plant pathogens, with effectiveness up to 48.28% and 55.17% respectively.

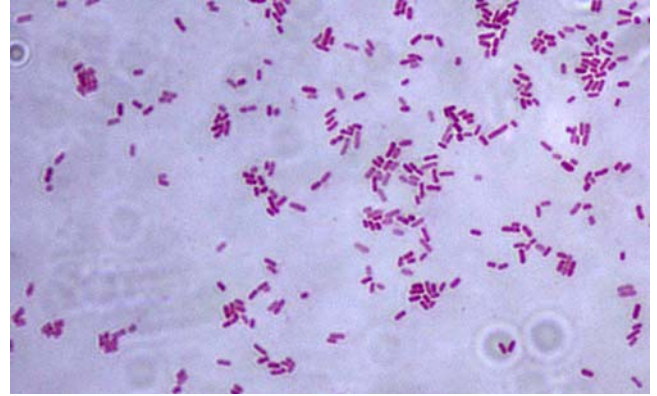


Figure 65. *Stenotrophomonas maltophilia*, in a genus that can be among the dominant bacteria associated with bryophytes in peatlands. Photo by Riraq25, through Creative Commons.

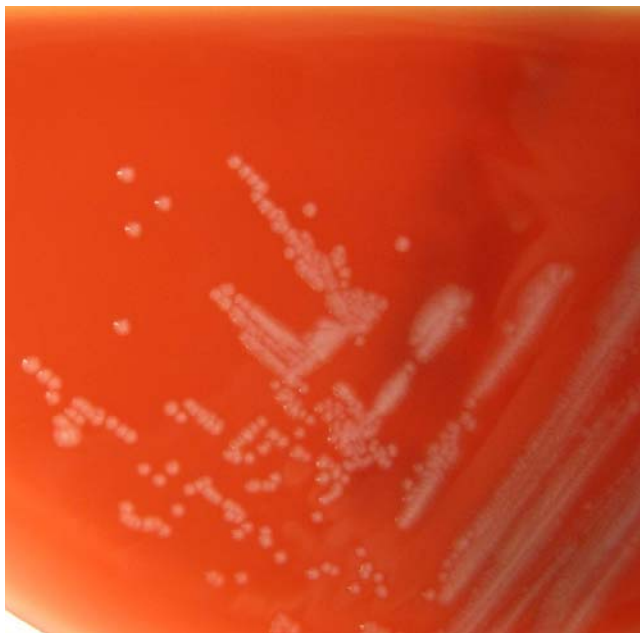


Figure 63. *Delftia*, a genus that can be among the dominant bacteria associated with bacteria. Photo by mostly harmless, through Creative Commons.



Figure 66. *Pectobacterium carotovorum*, a species that is inhibited by many bacterial isolates from bryophytes. Photo by Paul Bachi, through Creative Commons.

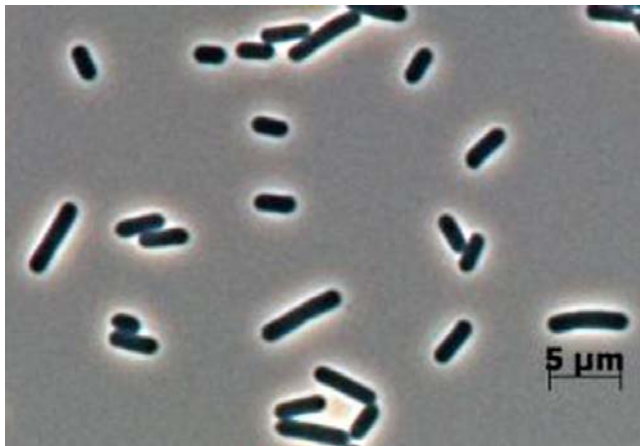


Figure 64. *Lysinibacillus*, a genus that can be among the dominant bacteria associated with bacteria. Photo from Leibniz-Institut DSMZ, through Creative Commons.

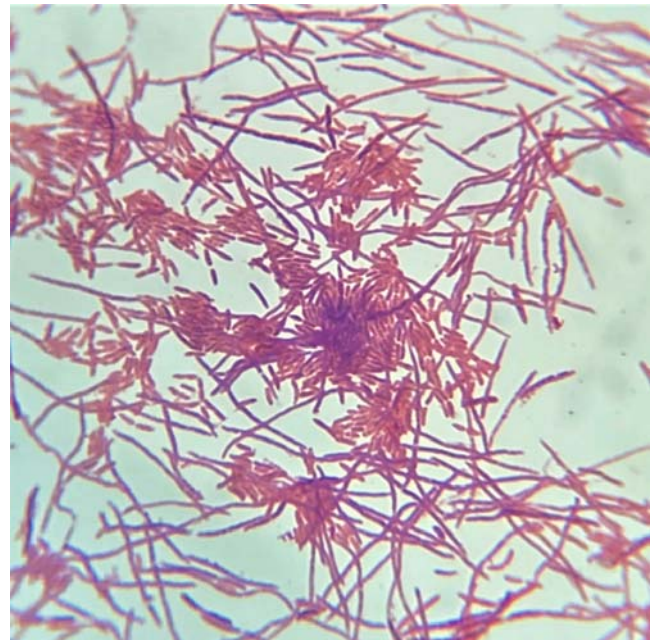


Figure 67. *Pseudomonas fluorescens* Gram stain, one of the most efficient bryophyte bacteria against plant pathogens. Photo by B. Domangue, through Creative Commons.



## Ecosystem Roles

Robroek *et al.* (2021) approached the "rewiring" of peatland plant-microbe interactions and communities using a network approach. They found that the prokaryotic communities differed between sites. This was manifest in a more rapid turnover in the plant-microbial interactions than in the communities they inhabited. They found that the turnover in the bacterial network composition was driven mostly by the establishment of new interactions between the plant community and that of the microorganisms, a phenomenon that was shared among all the sites.

Wicaksono *et al.* (2021a, b) produced evidence that the tracheophytes tended to have specific bacteria, whereas the bryophytes presented greater bacterial species richness and diversity. Nevertheless, Bragina *et al.* (2015) was able to demonstrate that the plants and lichens of bogs shared a core microbiome over the entire ecosystem, forming a transkingdom metacommunity. All of these bog organisms are connected to the keystone *Sphagnum* (Figure 49, Figure 54) species through the microbial species such as *Burkholderia bryophila* (see Figure 57). This bacterial species was associated with a wide array of host plants and provides a beneficial plant-microbe interaction.

Bragina *et al.* (2014) were among the early researchers attempting to unravel the roles of the symbiotic and protocoeoperation effects of the *Sphagnum* (Figure 49, Figure 54) microbiome. It appears that the microbiome is important in facilitating survival in the extreme conditions found in the *Sphagnum* habitats. The microbiome provides abundant subsystems that facilitate coping with oxidative and drought stresses, resistance to detrimental environmental factors, repair, and self-controlling mechanisms. Microbe-microbe and plant-microbe interactions are important in biofilm formation, interaction via quorum sensing (see Chapter 19-1 of this volume), and nutrient exchange. Their involvement in the nitrogen cycle and recycling of organic material are important contributions to the nutrient supply.

In addition to the products produced by the bacteria that are associated with *Sphagnum* (Figure 49, Figure 54), the bacteria can alter the response to a change in physical growth conditions as well. With the threat of global warming looming over the cold-climate peatlands, the ability of bacteria to rapidly acclimatize may promote the survival of *Sphagnum* through host-microbiome acquired thermotolerance (Carrell *et al.* 2021). First the researchers showed that elevated temperatures decreased the growth of sterile *Sphagnum* without addition of microbes. The addition of a microbiome from a thermal habitat matching the experimental temperatures returned the *Sphagnum* to its pre-warming growth rates. Warming changed the structure of the microbiome and induced a plant heat shock response. They suggested that the thermally conditioned microbiomes provided thermal conditioning to the *Sphagnum* host. The same results occurred when the microbiomes were isolated from *Sphagnum* warming experiments in Iceland, Sweden, and France.

But we may have barely scratched the surface of the interactions in the peatland community. As reported by Dedysh (2011) in her review, these uncultivated and unidentified bacteria form a large proportion of the microbial communities in acidic, cold, nutrient-poor, and water-saturated peatlands, hiding from us unknown physiologies and roles in the peatland ecology. New genetic techniques are permitting us to enumerate the number of strains present, and our culturing techniques are improving for these more elusive organisms.

## Decomposition

*Sphagnum* litter has a very slow decomposition, with first year loss of mass ranging 0.1-25% (Clymo 1965; Aerts *et al.* 2001; Scheffer *et al.* 2001; Dedysh 2011). This has been attributed to acidity (that favors fungi more than bacteria), phenolic compounds and waxes (mostly from shrubs) that are highly resistant to decay (Verhoeven & Liefveld 1997; Dedysh 2011), low nutrient content, especially N and P in *Sphagnum*, low temperatures, and anoxic conditions (Dedysh 2011). When decay occurs, the end-products are transferred into methane. This methane diffuses into the living, aerobic parts of the peat where Methanobacteria oxidize it and release CO<sub>2</sub>.

Bacteria affect the decay of *Sphagnum* (Figure 49, Figure 54), or more accurately, its near absence. Using *Sphagnum fallax* (Figure 49) from a fen woodland, Brock and Bregman (1989) found that after 12 months, the original N and P content associated with the moss had diminished little. Furthermore, the cells lacked much damage and had poor colonization by bacteria.

Patra (2020) reminded us of the role of *Sphagnum* (Figure 49, Figure 54) in secreting acids, making acid conditions that are unfavorable for the growth of most decomposing bacteria. The decomposition is further slowed by the absence of oxygen in the lower peat layers, causing an accumulation of dead material we know as peat.

## Xeric

Liu *et al.* (2014) investigated the endophytic bacterial community in the xerophytic moss *Grimmia montana* (Figure 68). Using a 212 sequence library, they identified 54 genera of bacteria in 4 phyla (*Pseudomonadota*, *Bacillota*, *Actinomycetota*, and *Cytophaga/Flexibacter/Bacteroids*). As in many other bryophyte bacterial communities, the *Pseudomonadota* were dominant (45.9%), with *Bacillota* being second (27.6%). The most abundant genera included *Acinetobacter* (Figure 69), *Aeromonas* (Figure 70), *Enterobacter* (Figure 71), *Leclercia*, *Microvirga*, *Paenisporosarcina*, *Planococcus*, *Planomicrobium*, *Pseudomonas* (Figure 13), and *Rhizobium* (Figure 53). They did not determine the role of the bacteria in the association, but some were known to have beneficial effects on their hosts. Some of the genera isolated differed from those detected by the molecular method, thus emphasizing that our methods may often miss important genera and species due to inappropriate culture conditions. At the same time, some taxa may not be in the molecular library.





Figure 68. *Grimmia montana* with capsules, a rock-dwelling species with 54 known genera of bacteria in its cells, spanning 4 phyla, with *Pseudomonadota* being dominant. Photo from Earth.com, with permission.

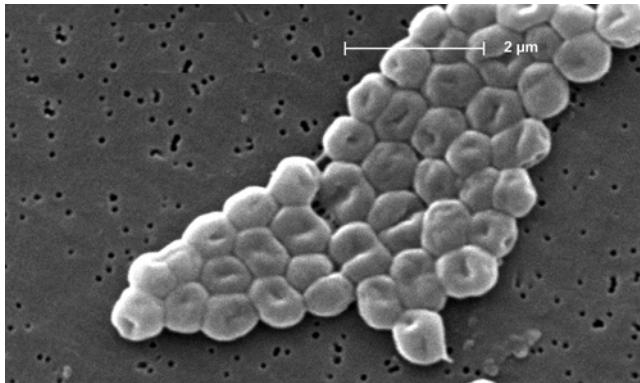


Figure 69. *Acinetobacter baumannii*, in one of the more abundant genera on *Grimmia montana*. Photo by Janice Carr, through Creative Commons.



Figure 70. *Aeromonas hydrophila*, in one of the more abundant genera on *Grimmia montana*. Photo by Nathan Reading, through Creative Commons.



Figure 71. *Enterobacter cloacae*, in one of the more abundant genera on *Grimmia montana*. Photo from CDC, through public domain.

### Soil Crusts

Soil crusts are a community of organisms that together colonize and stabilize soil surfaces. These are most common in dry areas where their ability to survive long periods of drought permit them to survive with little competition (Weber *et al.* 2019). These crusts occupy approximately ~12% of the terrestrial surface of the planet Earth (Weber *et al.* 2019). It is important that we understand these processes as they relate to ecosystem processes, especially in cryptogamic soil crusts (Deane-Coe & Stanton 2017), as our Earth remains in a state of continuous change.

The biocrusts may be dominated by **Cyanobacteria**, lichens, or bryophytes (Warren *et al.* 2019; Weber *et al.* 2019). The communities differ significantly from each other (Maier *et al.* 2018). Their organisms include free living, lichenized, and mycorrhizal fungi, **Cyanobacteria**, chemoheterotrophic bacteria, diazotrophic bacteria and archaea, eukaryotic algae, and bryophytes (Warren *et al.* 2019; Weber *et al.* 2019). The organisms are characterized by desiccation and extreme temperature tolerance, production of various soil-binding chemistries, almost exclusive dependency on asexual reproduction, pattern of aerial dispersal over impressive distances, and universal vulnerability to a wide range of human-related perturbations (Warren *et al.* 2019).

The role of bacteria in the bryophyte-lichen matrix of soil crusts remains poorly known. Weber *et al.* (2019) found that bacterial community composition changed in a stepwise manner along with biocrust succession, while bare soil communities were completely different. As the climate changes and land use destroys these communities, it is important that we understand the role of interactions, including those between the bacteria and bryophytes.

Weber *et al.* (2012) explored the soil crusts in the succulent Karoo of South Africa. They found that leaching from mosses may cause the high rates of soil respiration. The leaching creates microsites with high nutrient levels, favoring the growth of the microorganisms.



Moquin *et al.* (2012) examined the bacterial diversity in bryophyte-dominated soil crusts. They found the dominant phyla to be **Acidobacteriota**, **Bacteroidota**, and **Pseudomonadota**. Although tracheophytes are not a common member of the crusts, they found bacterial root associates, especially in the family **Oxalobacteraceae**. The presence of **Bacteroidota** suggests that the bacterial community in these crusts is affected by high carbon availability. The bacterial communities of soils and the **Cyanobacteria**-dominant crusts differed from those of the bryophyte-dominated ones. Bamforth (2008) noted that the microorganisms are important in the nutrient cycling of the crust community and subsequently contribute nutrients to the underlying soils.

In southwestern China, Cao *et al.* (2020) found that not only moss species, but also the types of karst rocky desertification, affect the microbial communities. Mosses were by far the more impacting factor, with changes in moss species bringing drought-resistant factors that affected the bacterial community, or *vice-versa*. The bacteria were able to provide proline content, superoxide dismutase activity, and peroxidase activity. These compounds are closely related to the drought adaptability of mosses.

Maier *et al.* (2018) found that alpha diversity of the crust community increases as succession progresses, with a concomitant shift from more generalized to more specialized organisms. At the same time, the CO<sub>2</sub> gas exchange exhibits significantly larger respiration rates in later successional stages. The NO and HONO emission patterns also change during succession. Thus, as the photosynthetic organisms change, they facilitate specific microbial communities, and these microbial changes in turn influence the physiological properties of the biocrusts and their contributions to both local and global nutrient cycles. The three dominance types of biocrusts have significantly different communities.

In the *Didymodon rigidulus* (Figure 72) community, there are endophytic bacteria, including *Bacillus cereus* (Figure 73), *Bacillus pumilus*, *B. subtilis* (Figure 10), *Bacillus* sp., *Neobacillus niacini*, *Peribacillus simplex* (see Figure 74), and *Priestia aryabhatai* (all previously in *Bacillus*; see Figure 9) (Ma *et al.* 2017b). The dominant species was *B. subtilis*. They found that at the test concentrations of petroleum ether, ethyl acetate, and n-butyl alcohol extracts, *B. cereus* and *B. subtilis* did not significantly alter the bryophyte spore germination. On the other hand, the protonema growth was inhibited to varying degrees by the same extracts. Hence, we know that bacteria can use their secondary metabolites to affect the development of bryophytes and alter the community composition.

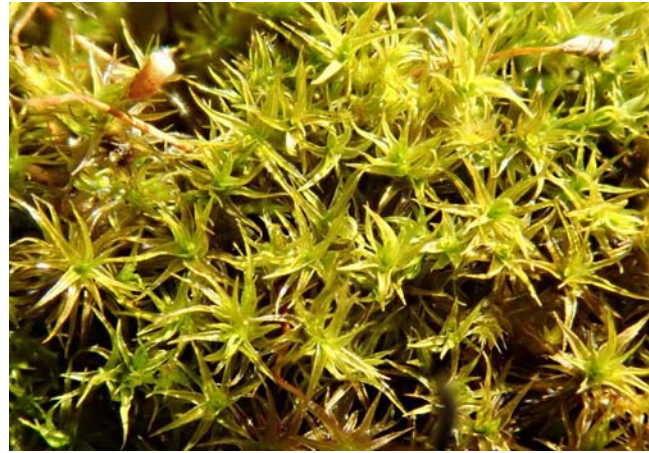


Figure 72. *Didymodon rigidulus*, a species with a number of endophytic bacteria; *Bacillus subtilis* is dominant. Hugues Tinguy, with permission.

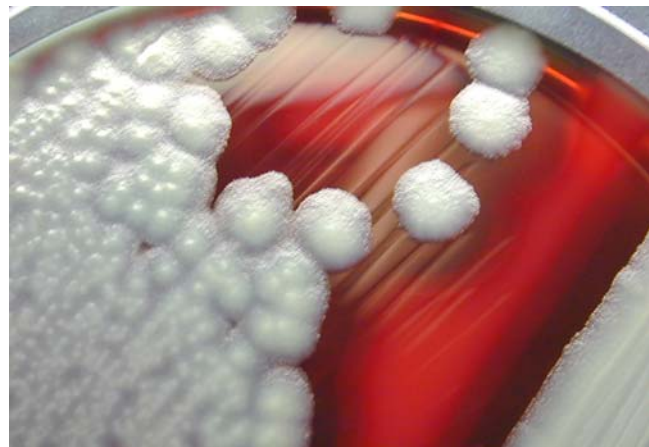


Figure 73. *Bacillus cereus* showing hemolysis on sheep blood; this is one of the endophytic bacteria found in *Didymodon rigidulus*. Photo by Larry Stauffer, through public domain.

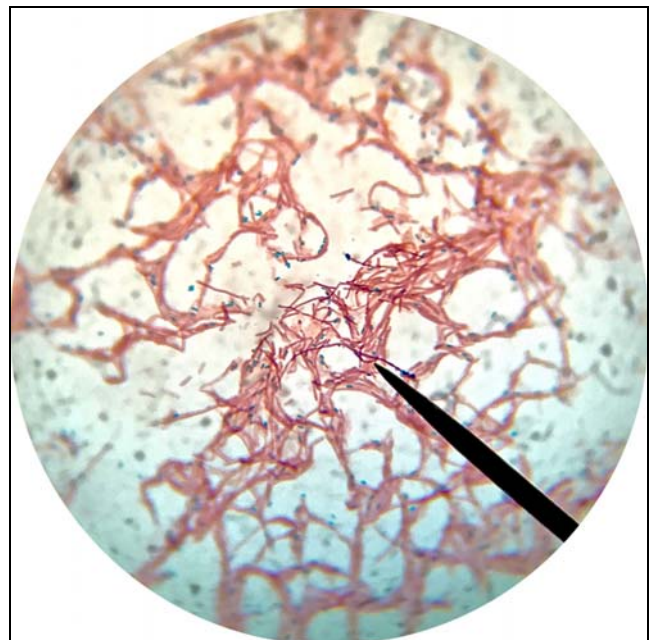


Figure 74. *Peribacillus subtilis* with endospores; *P. simplex* is one of the endophytic bacteria found in *Didymodon rigidulus*. Photo by W M Rapids, through Creative Commons.





## Honeybees

Honeybees can benefit from the antibacterial activity of bryophytes against bacteria. And without honeybees, the plant community can suffer due to absence of pollination. Gahtori *et al.* (2011) identified the bacterium *Medisaccoccus plutonius* as the cause of the European foulbrood disease in honeybees (Figure 75). They extracted antibacterial compounds from three different bryophytes and tested them against this bacterium. All of the tested extracts exhibited good antibacterial activity against the foulbrood bacteria. The maximum activity derived from *Dicranum undulatum* (Figure 76) and *Marchantia polymorpha* (Figure 17) and was comparable to that of the standard drug in use against these bacteria.



Figure 75. *Apis mellifera* (honeybee) on comb. Photo by A. Szalansk, through Creative Commons.



Figure 76. *Dicranum undulatum*, a species that has compounds that are antibacterial against the foulbrood bacteria in honeybees. Photo by Robin Bovey, with permission through Dale Vitt.

Sevim *et al.* (2017) tested the antibacterial activity of 23 bryophyte species in Turkey against *Paenibacillus larvae* (Figure 77) isolates from honeybee larvae; these bacteria are responsible for the American foulbrood diseases in the honeybee larvae. Of the 23 bryophytes sampled, they found 10 [liverwort *Metzgeria conjugata* (Figure 78); mosses *Calliergonella cuspidata* (Figure 79), *Calliergonella lindbergii* (Figure 80), *Grimmia alpestris* (Figure 81), *Isoetecium alopecuroides* (Figure 82),

*Polytrichastrum formosum* (Figure 83), *Polytrichum commune* (Figure 84), *Syntrichia calcicola* (Figure 85), *Syntrichia intermedia* (Figure 86), *Tortella densa* (Figure 87)] that exhibited good antimicrobial activity against *P. larvae* isolates.



Figure 77. *Paenibacillus larvae*, a bacterium in honeycombs that affects the larvae of the honeybee. Photo from Georgia Department of Agriculture, through Creative Commons.



Figure 78. *Metzgeria conjugata*, a liverwort that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Hermann Schachner, through Creative Commons.



Figure 79. *Calliergonella cuspidata*, a wetland moss that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Hugues Tinguy, with permission.





Figure 80. *Calliergonella lindbergii*, a wetland moss that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Hermann Schachner, through Creative Commons.



Figure 83. *Polytrichastrum formosum*, a soil-dwelling moss that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Kent Brothers, Botany Website, UBC, with permission.



Figure 81. *Grimmia alpestris*, a rock-dwelling moss that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Hermann Schachner, through Creative Commons.



Figure 84. *Polytrichum commune*, a wetland moss that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Riken Mon, through Creative Commons.



Figure 82. *Isoetecium alopecuroides*, a rock-dwelling moss that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Hermann Schachner, through Creative Commons.



Figure 85. *Syntrichia calcicola*, a xerophytic moss that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Hermann Schachner, through Creative Commons.





Figure 86. *Syntrichia intermedia*, a xerophytic moss that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Acta Plantarum, through Creative Commons.



Figure 87. *Tortella densa*, a moss of mostly rather dry habitats that makes compounds that are effective against the bacterial American foulbrood disease in honeybee larvae. Photo by Hermann Schachner, through Creative Commons.

But does this protection work in nature? Are honeybees able to bring mosses to the hive to prevent the growth of the bacteria? Do they have behavioral adaptations that cause them to move about among the mosses to sanitize themselves? What an interesting relationship it would be if such activities were true. But we don't know; perhaps nature was simply waiting for humans to do the sanitizing job.

### Pollution Relationships

Meyer *et al.* (2010a) compared bryophyte microbial communities in rural, urban, and industrialized communities. The particulate atmospheric pollution affects the bryophyte-microorganism complexes. They found that microalgae, bacteria, rotifers, and testate amoebae biomasses were significantly higher in the rural site. The physico-chemical variables ( $\text{NO}_2$ , relative humidity,

temperature, site) and the trace elements accumulated in bryophytes (copper, strontium, lead) explained 69.3% of the variance in the microbial community. These numbers suggest that bacteria in a community are potential biomonitors of atmospheric pollution.

Rojas *et al.* (2016) reported that bryophytes dominated control precipitates, whereas the **Basidiomycota** fungi were most abundant under reclaimed precipitates at an acid mine drainage reclamation site in central Pennsylvania, USA. Furthermore the reclaimed precipitates had more bacterial diversity than did the controls. Bacteria under bryophytes were more common under unreclaimed (control) soils.

Meyer *et al.* (2010b) found that the bryophytes did not accumulate the low concentrations of metallic trace elements. However, the **Cyanobacteria**, testate amoebae, and fungi all decreased in the microbial community in response to the particle deposition. Thus, the composition of the microbe community could serve as a useful indicator of pollution effects.

### Reclamation Communities

Bryophytes can help to reclaim despoiled areas in a number of ways. Kyyak *et al.* (2020) found that they could colonize saline substrates of tailings storage, where they provide extended surface area for the accumulation of organic matter. The organic carbon under moss turfs increased 2.2-5.0 times compared to areas with no bryophytes. The dense-turf mosses *Didymodon rigidulus* (Figure 72) and *Ptychostomum pseudotriquetrum* var. *bimum* (Figure 88) accumulated the most organic matter, compared with loose turf formed by *Barbula unguiculata* (Figure 89) and *Funaria hygrometrica* (Figure 90). The moss turfs facilitate a 0.2-0.5 unit increase in acidity of the aqueous solutions of the tailings. Bacterial quantity increased significantly under mosses in reclaimed mine tailings, achieving a biomass under the moss turfs of *Didymodon rigidulus* and *Funaria hygrometrica* of 5.09-6.10  $\mu\text{g C g}^{-1}$  dry weight soil. Without mosses, bacteria reached only 3.19-11.27  $\mu\text{g C g}^{-1}$  dry weight soil.



Figure 88. *Ptychostomum pseudotriquetrum* var. *bimum* where organic matter accumulates in greater amounts than in the tailings beneath the loose moss turfs. Photo by Hugues Tinguy, with permission.





Figure 89. *Barbula unguiculata* dry, where less organic matter accumulates in the tailings beneath the moss loose turfs than in dense turf areas. Photo by Bob Klips, with permission.



Figure 90. *Funaria hygrometrica* where less organic matter accumulates in the tailings beneath the moss loose turfs than in the dense turf areas. Photo by Kurt Stueber, through Creative Commons.

The microbial biomass in the tailings beneath the moss turfs depended on the species characteristics of the mosses (Kyyak *et al.* 2020). The microbial biomass index almost doubled with the high level of salinization under turfs of *Didymodon rigidulus* (Figure 72) and *Funaria hygrometrica* (Figure 90). The moss cover also promoted a significant increase in the primary ecological and trophic groups of microorganisms (saprophytes, cellulose-degrading bacteria, oligonitrophils, and nitrogen fixers). Thus the pioneer bryophytes are important in the accumulation of organic matter, increase in acidity, improvement of the redox regime of the substrate, and promotion of development of the important soil microbiota (DeLuca *et al.* 2002; Gavazov *et al.* 2010; Stewart *et al.* 2011; Kyyak *et al.* 2020). In particular, they increased the productivity of cellulose-degrading bacteria.

## Summary

It is only in the 21st century that much research has addressed the bacterial communities associated with bryophytes. It appears that these communities have important roles in the ecosystems they inhabit.

Bryophytes in rivers and streams trap organic sediments. These provide rich habitats for bacteria, which in turn provide food for invertebrates both large and small. We now know that they can cause denitrification, whereas others are important in nitrogen fixation, while at the same time converting methane to free CO<sub>2</sub> that is used by the bryophytes. Their role in nitrogen fixation in peatlands, the boreal forest, cryptogamic crusts, and other low-nutrient habitats is significant. They are also, at least in part, responsible for degradation, senescence, and decomposition of the bryophytes, thus contributing to nutrient cycling. However, in acidic peatlands, the decomposition is slow.

Some bacteria produce ice-binding proteins that help bryophytes, especially in the polar and alpine regions, to survive freezing by preventing large crystals from forming in the bryophyte cells.

Some communities are very similar on a number of bryophyte species in an area, while at the same time some bryophytes can have unique communities. But we do not understand what causes the bacterial community differences with bryophyte species – microhabitat needs, bryophyte secondary compounds, bryophyte structure, bacteria-bacteria interactions?

There are many habitats where the bacterial associates of bryophytes have not been assessed. Their roles in these communities could be critical for some of the vital ecosystem functions. It is likely that there are multiple connections for some of these roles and that the community composition will change with climate change, probably before we begin to understand these connections.

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# **CHAPTER 19-3**

## **BRYOPHYTE DEFENSES AGAINST BACTERIA**

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# CHAPTER 19-3

## BRYOPHYTE DEFENSES AGAINST BACTERIA



Figure 1. *Salmonella typhi*, a human pathogen that is very sensitive to bryophyte extracts. Photo from CDC, through public domain.

### Defenses Against Bacteria

Bryophytes generally seem to lack damage by bacteria and other pathogens. Although some bacteria can be pathogens on bryophytes, others actually help to protect the bryophytes.

Martínez-Abaigar and Núñez-Olivera (2021) referred to bryophyte defenses as "the outstanding capacity of bryophytes to produce bioactive compounds with diverse biological functions." In addition to the great variety of terpenoids produced by liverworts, all three bryophyte lineages can produce phenolic derivatives (from simple cinnamic acids to complex flavonoids), alkaloids, and lipids. Among these defenses, the liverwort bisbibenzyls and sesquiterpenoid derivatives and the moss diterpenoid derivatives momilactones are the most important compounds.

We now know that bryophyte defenses include phenylquinone, aromatic and phenolic substances, oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, and aliphatic compounds (Alam *et al.*

2012). Bryophytes also produce polyunsaturated C<sub>20</sub> fatty acids. These include arachidonic acid and eicosapentaenoic acid (Ponce de León *et al.* 2015). These can be oxidized and transformed into bioactive compounds.

More than 1600 terpenoids have been identified from bryophytes (Chen *et al.* 2018). Some of these are unique to bryophytes. These terpenoids have a variety of functions, but they are particularly useful as defenses against both biotic and abiotic stresses.

Until 2016 momilactones were known only from rice and the moss *Hypnum plumaeforme* (Figure 2) (Okada *et al.* 2016). These compounds are diterpenoid phytoalexins with antimicrobial and allelopathic functions. A similar transcription response to stresses was identified in *Physcomitrium patens* (Figure 3), suggesting a similarity between mosses and tracheophytes in response to stresses, including pathogens. On the other hand, jasmonic acid seems to be absent in bryophytes, whereas it is a signalling mechanism in tracheophytes, initiating plant defenses (Ponce de León *et al.* 2015; Okada *et al.* 2016).





Figure 2. *Hypnum plumaeforme* moist, source of momilactones that are antibiotic and allelopathic. Photo by Janice Glime.



Figure 3. *Physcomitrium patens*, a new source of momilactones. Photo by Michael Lüth, with permission.

Liverworts seem to have the most diverse array of secondary compounds with antibiotic properties (Russell 2010). Among the 14 species tested, 88% had antibiotic activity, whereas only 33% of the moss species exhibited any activity against the three bacterial strains tested. The liverwort *Lunularia cruciata* (Figure 4) presented the greatest activity against the three bacteria tested. But this is only a small sampling of bacteria and bryophytes. A wider array of bacterial species would most likely reveal even more kinds of activity.

The liverworts are known for their often distinctive odors. They also have distinctive **oil bodies** in the cells, and these are unique enough that they are often of taxonomic value. We now know that these oil bodies are the sites of many defense compounds – secondary compounds that seem to have no other metabolic functions (Asakawa 2011). The oil bodies are known only from the liverworts and are the site for storing terpenoids (He *et al.* 2013). These include the mono-, sesqui- and di-terpenoids, aromatic compounds like bibenzyl, bis-bibenzyls, and acetogenins (Asakawa *et al.* 2013) – compounds that serve

to protect the liverworts against both herbivory and pathogens (antimicrobial, antifungal, and antiviral). On the other hand, mosses and hornworts produce primarily di- and triterpenes (Zhan *et al.* 2015). Among the liverworts, more than 40 new carbon skeletons of terpenoids and aromatic compounds have been found (Asakawa & Ludwiczuk 2017).



Figure 4. *Lunularia cruciata*, a species with the greatest antibacterial activity among species in one test. Photo from Botany Website, UBC, with permission.

Wang *et al.* (2006) suggested that the lower inhibitory activity of *Cylindrocolea recurvifolia* (Figure 5) compared to that of *Pleurozia subinflata* (Figure 6), both leafy liverworts, could be due to the lower contents of oil bodies in the former, where they are both smaller and fewer. On the other hand, Zhu *et al.* (2006) found that there was no correlation between antibacterial activity and size or number of oil bodies in 38 liverwort species.



Figure 5. *Cylindrocolea recurvifolia*, a leafy liverwort with small and few oil bodies and less antibiotic activity than that of *Pleurozia subinflata*. Photo from Museum of Hiroshima University, with permission.



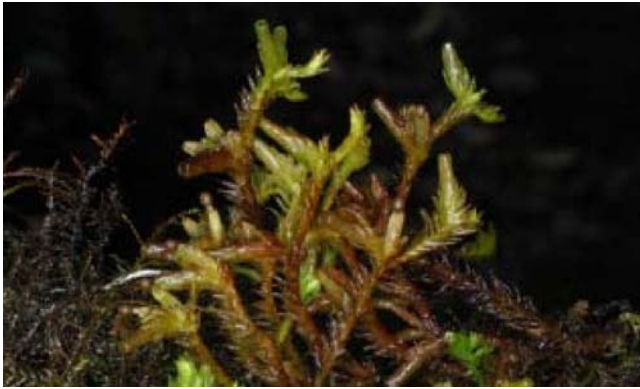


Figure 6. *Pleurozia subinflata*, a leafy liverwort with larger and more numerous oil bodies than those of *Cylindrocolea recurvifolia* and with greater antibiotic activity. Photo by Jan-Peter Frahm, with permission.

Mosses and liverworts seem to lack tissue-specific antibody binding against the lignin-like polymers homoguaiacyl (G) and guaiacyl/syringyl (GS) (Ligrone *et al.* 2008). On the other hand, the hornworts *Megaceros pellucidus* (Figure 7) and *Nothoceros fuegiensis* (see Figure 8) exhibited more intense labelling with the GS antibody of the pseudoelaters and spores than in the other cell types.



Figure 7. *Megaceros pellucidus*, a species in which GS antibody labelling of the pseudoelaters and spores was greater than for other cell types. Photo by Ashley Bradford, through Creative Commons.



Figure 8. *Nothoceros aenigmaticus*, a species in which GS antibody labelling of the pseudoelaters and spores was greater than for other cell types. Photo by Juan Carlos Villareal, with permission.

## Antibiotic Response by Bryophytes

Banerjee and Sen (1979) reported that 56% of the bryophytes they tested were active against at least one bacterial species. Our knowledge of moss antibiotic properties is much less than that of liverworts (Provenzano *et al.* 2019). Only 3.2% of the mosses and 8.8 of the hornworts have been characterized. This is probably because the liverworts seem to a more promising variety of interesting secondary compounds that could be useful to humans.

Van Hoof *et al.* (2013) found that the moss *Hypnum cupressiforme* (Figure 9) had strong antimicrobial effects. In this case, at least, the activity was greater against plant bacteria than it was against human bacteria.



Figure 9. *Hypnum cupressiforme*, a species with strong antibacterial effects against plant bacteria. Photo by J. C. Schou, with permission.

Sawant and Karadge (2010) found that extracts of the cave liverwort *Cyathodium cavernarum* (Figure 10) were mostly inactive against the bacteria tested, whereas other liverworts [*Plagiochasma intermedium* (Figure 11), *Asterella wallichiana* (Figure 12), *Targionia hypophylla* (Figure 13)] in these tests exhibited good antimicrobial activity. Chavhan (2017) likewise found that *Targionia hypophylla* exhibited a high level of antibiotic activity against two bacterial strains. And *Cyathodium tuberosum* (Figure 14) exhibited the least. Is there a pattern to the absence of antimicrobial properties in cave mosses? This would seemingly save energy in these low-energy systems.



Figure 10. *Cyathodium cavernarum*, a thallose cave liverwort that doesn't seem to possess antibodies. Photo by Cédric de Foucault, through Creative Commons.





Figure 11. *Plagiochasma intermedium*, a liverwort species with good antibiotic activity. Photo from Earth.com, with permission.



Figure 12. *Asterella wallichiana* with young archegonial heads, a liverwort species with good antibiotic activity. Photo by Shyamal L., through Creative Commons.



Figure 13. *Targionia hypophylla*, a liverwort species with good antibiotic activity. Photo by Hugues Tinguy, with permission.



Figure 14. *Cyathodium tuberosum* a liverwort with the weakest antibiotic activity among those tested. Photo by Silvia Pressel and Jeff Duckett, with permission.

Zhu *et al.* (2006) found that 93% of the 60 tested bryophytes exhibited antibacterial activity. All liverworts tested (38) had activity against at least two bacterial species tested. Of the 60 bryophyte species, 17 were active against all seven of the tested bacterial species (Gram positive: *Bacillus megaterium* (Figure 15), *Bacillus subtilis* (Figure 16), *Bacillus thuringiensis* (Figure 17), *Staphylococcus aureus* (Figure 18); Gram negative: *Escherichia coli* (Figure 19), *Pseudomonas aeruginosa* (Figure 20), *Pseudomonas putida*). The activity was especially high in the liverwort genera *Conocephalum* (Figure 21), *Frullania* (Figure 22), *Herbertus* (Figure 23), *Marchantia* (Figure 24), *Mastigophora* (Figure 25), and *Porella* (Figure 26). Among these, *Staphylococcus aureus* was the most resistant to bryophyte extracts from both mosses and liverworts, in sharp contrast to a number of other studies in which it was the most susceptible to bryophyte extracts (*e.g.* Bodade *et al.* 2008; Liu & Wang 2010; Liyanage *et al.* 2015; Sabovljević *et al.* 2010). The most sensitive bacterial species to moss extracts was *Pseudomonas putida*; sensitivity to liverwort extracts was greatest in *Bacillus subtilis*. When negative results are found, it is possible that the bryophyte had not received the proper signals to make the antibiotic compounds. This would be particularly true in sterile cultures.



Figure 15. *Bacillus megaterium*, one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo by Alexastely, through Creative Commons.



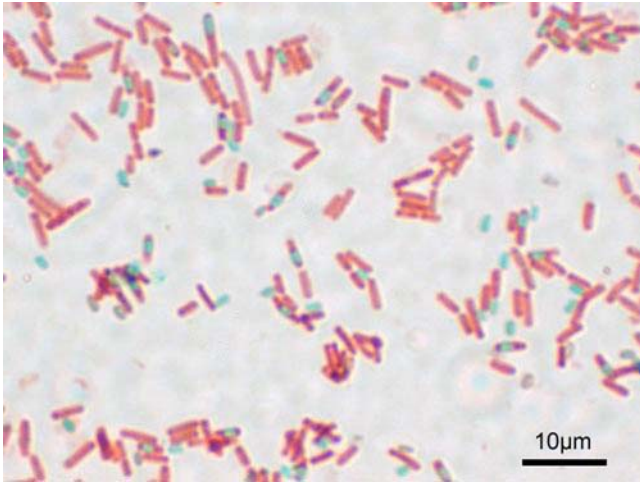


Figure 16. *Bacillus subtilis* forming spores, one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo by Y. Tambe, through Creative Commons.



Figure 17. *Bacillus thuringiensis*, one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo by Todd Parker, CDC, through public domain.

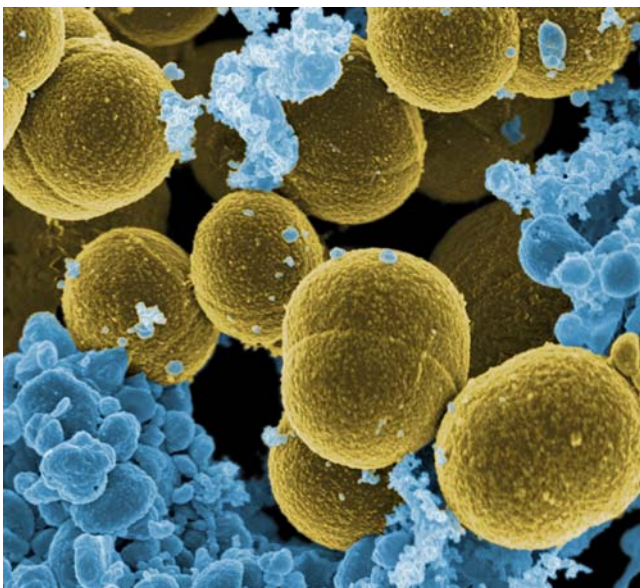


Figure 18. *Staphylococcus aureus*, one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo from NIAID-RML, through public domain.

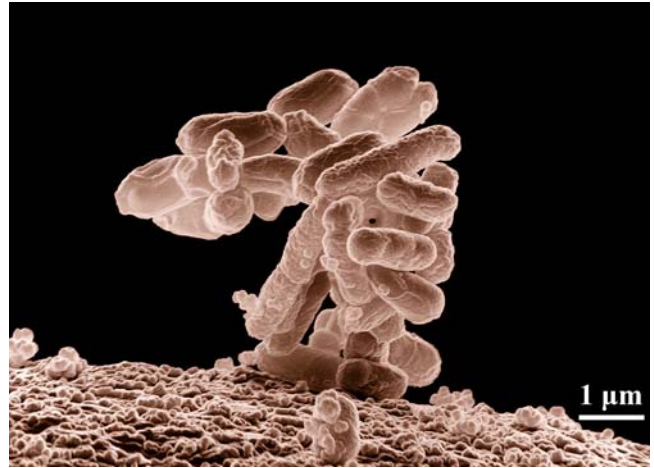


Figure 19. *Escherichia coli*, one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo by Erbe, digital colorization by Christopher Pooley, both of USDA, ARS, EMU, through public domain.



Figure 20. *Pseudomonas aeruginosa*, a bacterial species resistant to multiple drugs and one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo by Jennifer Oosthuizen, CDC, through public domain.



Figure 21. *Conocephalum conicum* from the UK, in a genus with especially high antibacterial activity. Photo by Lairich Rig, through Creative Commons.





Figure 22. *Frullania dilatata*, in a genus with especially high antibacterial activity. Photo by Paul Bowyer, through Creative Commons.



Figure 25. *Mastigophora woodsii*, in a genus with especially high antibacterial activity. Photo by Claire Halpin, with permission.



Figure 23. *Herbertus aduncus*, in a genus with especially high antibacterial activity. Photo from Earth.com, with permission.



Figure 26. *Porella platyphylla*, in a genus with especially high antibacterial activity. Photo by Hermann Schachner, through Creative Commons.



Figure 24. *Marchantia polymorpha*, a species that responds to the bacterium *Pseudomonas syringae* by producing antibacterial compounds. Image copyright Stuart Dunlop <[www.donegal-wildlife.blogspot.com](http://www.donegal-wildlife.blogspot.com)>, with permission.

When Shirzadian and Afshari Azad (2010) tested the activity of 11 Iranian bryophytes, they found that only *Xanthomonas citri* pv. *malvacearum* (pv. = pathovar; Figure 27) failed to show any response to extracts from the bryophytes. The other bacteria [*Erwinia amylovora* (Figure 28), *Pectobacterium carotovora* (Figure 29), *Ralstonia solanacearum* (Figure 30), *Agrobacterium tumefaciens* (Figure 31)] responded to the extracts.

It is interesting that the  $\alpha$ -DOX ( $\alpha$ -dioxygenase) in the moss *Physcomitrium patens* (Figure 3) is both part of the defense system and a controlling agent in development (Machado *et al.* 2015).  $\alpha$ -DOX contributes to the synthesis of **oxylipins**, permitting plant signaling against both biotic and abiotic stresses. On the other hand, Bressendorff *et al.* (2016) found that the moss *Physcomitrium patens*, unlike tracheophytes, uses a different signalling pathway for immunity than the one used to respond to osmotic stress.

The evolution in bacteria only got part way to having a successful jasmonic acid defense (Monte *et al.* 2018). They have the genes for the JA-Ile (jasmonoyl-isoleucine) signalling pathway, but they do not produce JA-Ile.





Figure 27. *Xanthomonas citri* pv *malvacearum* on cotton leaf, a bacterium that failed to respond to bryophyte extracts from Iran. Photo from Clemson University - USDA Cooperative Extension Slide Series, through Creative Commons.



Figure 28. *Erwinia amylovora* on apples, a bacterium that was inhibited by extracts from Iranian bryophytes. Photo from University of Georgia Plant Pathology, University of Georgia, <Bugwood.org>, through Creative Commons.



Figure 29. *Pectobacterium carotovora* on elm, a bacterium that was inhibited by extracts from Iranian bryophytes. Photo by Ninjatacoshell, through Creative Commons.



Figure 30. *Ralstonia solanacearum* wilt symptoms; this bacterium was inhibited by extracts from Iranian bryophytes. Photo from Clemson University - USDA Cooperative Extension Slide Series - USDA Forest Service, through Creative Commons.



Figure 31. *Agrobacterium tumefaciens*, a bacterium that was inhibited by extracts from Iranian bryophytes. Photo by William Jacobi, Colorado State University, <Bugwood.org>, through Creative Commons.

### Habitat Differences?

It seems reasonable to hypothesize that bryophytes from damp habitats are more likely to provide a suitable habitat for bacteria and fungi. Therefore, we can also hypothesize that bryophytes of moist habitats should have more defense compounds than those from dry habitats. Or could these be obtained through partnerships?

Liu and Wang (2010) noted that the moss *Ditrichum pallidum* (Figure 32) was able to defend against the bacteria *Staphylococcus aureus* (Figure 18), *Escherichia coli* (Figure 19), and *Proteus vulgaris* (Figure 33) to different degrees. Could this indicate differences in abundance of these three bacteria in the habitat of the



*Ditrichum pallidum* used in the tests? Or is it just specificity to the individual species and their relative abundance in bryophyte habitats?



Figure 32. *Ditrichum pallidum*, a moss that has different degrees of response to bacteria, depending on the bacterial species. Photo by Hugues Tinguy, with permission.

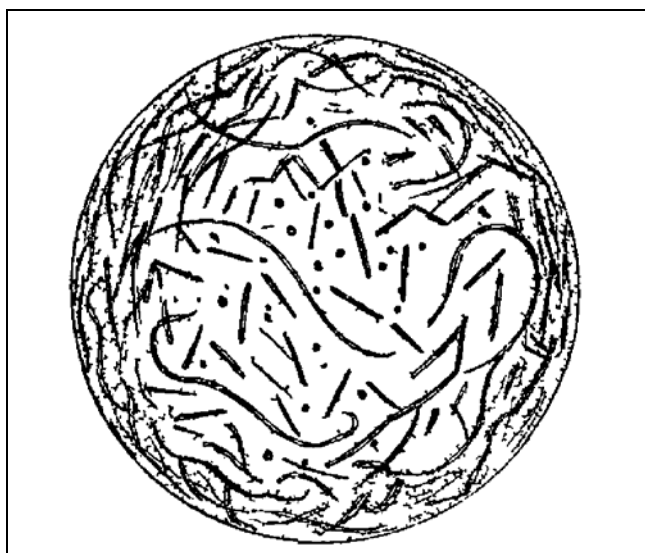


Figure 33. *Proteus vulgaris* drawing, the species that suffered the least effect by *Ditrichum pallidum* extracts in tests by Liu and Wang (2010). Image from Project Gutenberg Distributed Proofreaders, Encyclopædia Britannica, 1911, through public domain.

Bodade *et al.* (2008) similarly found that the dry habitat moss *Racomitrium crispulum* (Figure 34) did not provide any effective antibacterial compounds against the bacteria tested [including *Escherichia coli* (Figure 19) and *Staphylococcus aureus* (Figure 18)], whereas other bryophyte species in the test were all effective at least some of the time.

Dulger *et al.* (2005) tested 8 mosses from relatively dry habitats of rocks, soil, and tree trunks in Turkey and found that they inhibited 11 species of bacteria. The most susceptible bacteria among these were *Bacillus subtilis* (Figure 16) and *Pseudomonas aeruginosa* (Figure 20). The antiyeast activity was weak.



Figure 34. *Racomitrium crispulum* with capsules, an exposed-rock moss that was ineffective against the tested bacteria. Photo by Larry Jensen, with permission.

*Leptodictyum riparium* (Figure 35), a moss of wet habitats, had the best inhibitory power against all eight bacteria tested when compared to that of the thallose liverwort *Conocephalum conicum* (Figure 21) and the moss *Plagiomnium undulatum* (Figure 36) (Castaldo-Cobianchi *et al.* 1988). They commented there is competition between species growing in the water where one might find *L. riparium*.



Figure 35. *Leptodictyum riparium*, a moss that is even more inhibitory toward eight bacteria than the strongly inhibitory *Conocephalum conicum*. Photo by Hermann Schachner, through Creative Commons.



Figure 36. *Plagiomnium undulatum*, a moss that is less inhibitory toward eight bacteria than the strongly inhibitory *Leptodictyum riparium*. Photo by Hermann Schachner, through Creative Commons.



Altuner *et al.* (2014) found that the antibacterial activity of the forest floor species *Dicranum polysetum* (Figure 37) was especially strong against *Staphylococcus carnosus* (see Figure 18). It is interesting that such a strong activity against *Staphylococcus carnosus* exists when this bacterial species is not known from any natural habitat **and** it has no known pathogenicity (Löfblom *et al.* 2017)! Furthermore, it lacks any pathogenicity genes. Altuner *et al.* (2014) found that all three mosses in their study [*Calliergonella cuspidata* (Figure 38), *Dicranum polysetum*, and *Hypnum cupressiforme* (Figure 9)] were active against several species of Gram positive and Gram negative pathogenic bacteria.



Figure 37. *Dicranum polysetum*, a species with especially strong antibacterial activity against the non-pathogenic *Staphylococcus carnosus*. Photo by Kristian Peters, through Creative Commons.



Figure 38. *Calliergonella cuspidata*, a species active against several Gram positive and Gram negative pathogenic bacteria. Photo by Claire Halpin, with permission.

Dey *et al.* (2015) found that elevation made a difference in antibacterial activity of *Pellia endiviifolia* (Figure 39) from the eastern Himalayas. Those collected at

higher elevations had significantly higher antimicrobial activity. They suggested this might be due to differences in UV light levels, with the intensity increasing at higher elevations.



Figure 39. *Pellia endiviifolia*, a species with significantly higher antibacterial activity at higher altitudes. Photo by David T. Holyoak, with permission.

By contrast, Mukherjee *et al.* (2012) found that in the thallose liverwort *Dumortiera hirsuta* (Figure 40) those specimens from a higher elevation had slightly lower antibacterial activity than those from lower elevations. Clearly more detailed information is needed about the habitats to determine the differences in antibacterial activity.



Figure 40. *Dumortiera hirsuta*, a species with slightly lower antibacterial properties at higher elevations. Photo by Mutolisip, through Creative Commons.

Out of 29 species of bryophytes from Sri Lanka, only *Pogonatum marginatum* (a species of wet soil and shady banks; Figure 41) failed to respond with antibiotics against any of the test bacteria [*Lysinibacillus sphaericus* (MTCC511), *Staphylococcus aureus* (Figure 18) (ATCC25923), *Klebsiella pneumoniae* (Figure 42) (ATCC700603), *Pseudomonas aeruginosa* (Figure 20) (ATCC27853)] (Liyanage *et al.* 2015).





Figure 41. *Pogonatum urnigerum*; *Pogonatum marginatum* from Sri Lanka failed to produce any antibiotic in response to test bacteria. Photo by Hermann Schachner, through Creative Commons.

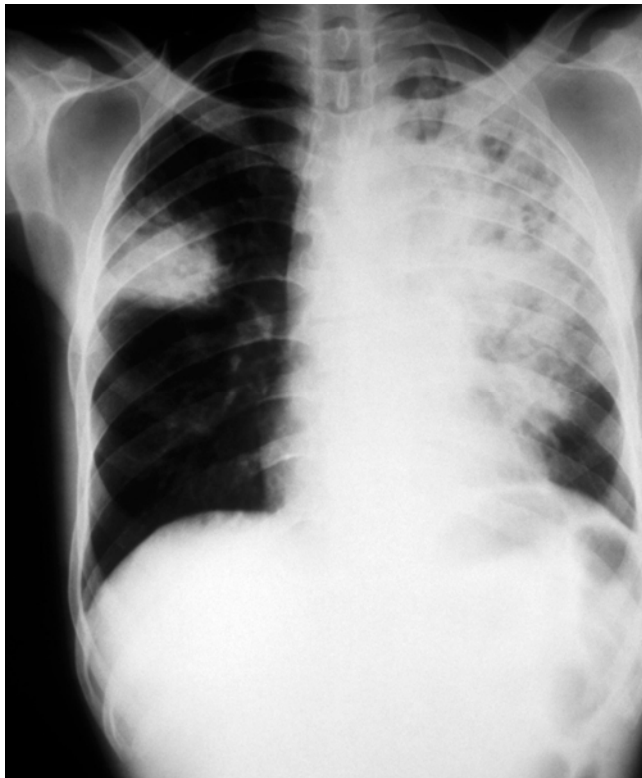


Figure 42. Human lung X-ray showing damage by *Klebsiella pneumoniae*, especially in left lung; *Pogonatum marginatum* fails to respond to this bacterium. Photo through Creative Commons.

### Bacterial Defense Partners

Bacteria themselves can often be of antibiotic benefit to the bryophytes. They can provide antagonistic compounds that serve as defense compounds against other bacteria and fungi. I wonder if the bacteria ever serve as deterrents to larger herbivores such as insects, birds, and rodents. One would expect the insects to avoid some of the bryophytes because they produce insect repellent (Ludwiczuk & Asakawa 2019), but it seems that the bacteria could also serve this role.

Mechanisms of control of microorganisms by bryophyte-associated bacteria include secretion of metabolic substances (e.g. antibiotics, **siderophores** - small, high-affinity iron-chelating compounds secreted by microorganism), controlling proliferation, and competitive exclusion of plant pathogens (Glick & Bashan 1997; Muleta *et al.* 2007; Szentes *et al.* 2010). Among these antagonistic bacteria Szentes *et al.* (2010) found the genera *Azospirillum* (Figure 43), *Bacillus* (Figure 15, Figure 16, Figure 17, Figure 74), *Burkholderia* (Figure 44), *Enterobacter* (Figure 45), *Pseudomonas* (Figure 20), and *Rhodococcus* (Figure 46).



Figure 43. *Azospirillum* promoting root hair growth (**upper**) compared to roots with no *Azospirillum* (**lower**). Members of this genus control proliferation and facilitate competitive exclusion of plant pathogens that occur on bryophytes. Photo by T. A. Toennisson, through Creative Commons.

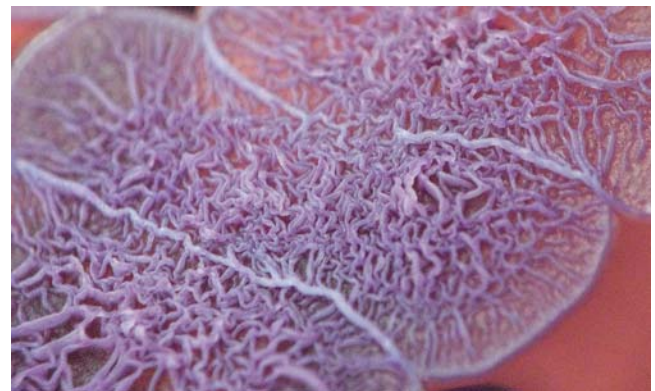


Figure 44. *Burkholderia thailandensis*; members of this genus control proliferation and facilitate competitive exclusion of plant pathogens that occur on bryophytes. Photo through Creative Commons.





Figure 45. *Enterobacter cloacae*; members of this genus control proliferation and facilitate competitive exclusion of plant pathogens that occur on bryophytes. Photo from CDC, through public domain.

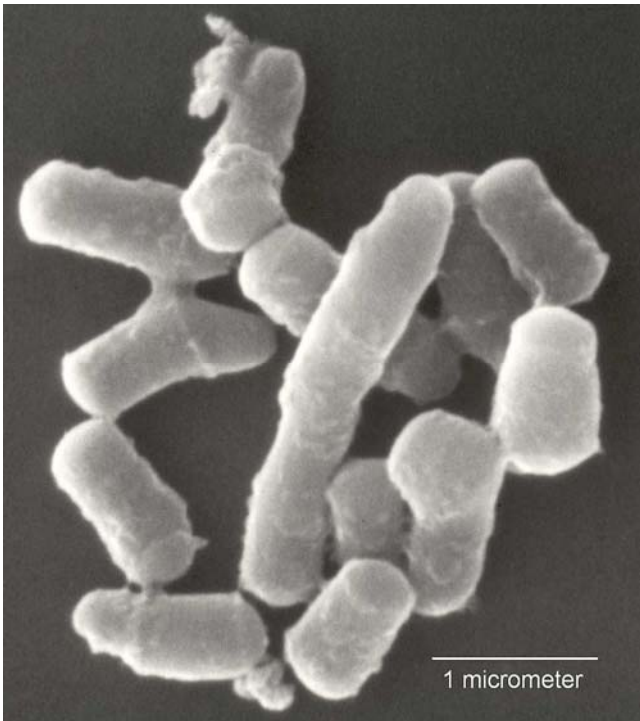


Figure 46. *Rhodococcus*; members of this genus control proliferation and facilitate competitive exclusion of plant pathogens that occur on bryophytes. Photo by Jerry Sims, through public domain.

Banerjee and Sen (1979) found that the liverwort family **Reboulia** (Figure 47) had especially good antibiotic activity in all 5 tested species. The moss *Brachythecium procumbens* and the liverworts *Asterella wallichiana* (Figure 48) and *Marchantia paleacea* (Figure 49) showed the widest range of antibiotic activity. *Salmonella typhi* (Figure 1) was the most sensitive of the microorganisms used in the tests.



Figure 47. *Reboulia hemisphaerica*, in a family (**Reboulia**) with especially good antibiotic activity. Photo by Hermann Schachner, through Creative Commons.



Figure 48. *Asterella wallichiana* with young archegonial heads, among the species with the widest range of antibiotic activity. Photo by Shyamal L., through Creative Commons.



Figure 49. *Marchantia paleacea*, among the bryophyte species with the widest range of antibiotic activity. Photo by Des Callaghan, with permission.

It is interesting that some newly recognized strains of bacteria present among the bryophytes have toxicity to things that presumably never affect the bryophytes. For



example, 12 new strains of *Bacillus thuringiensis* (Figure 17) were isolated from among 76 bryophyte species (Zhang *et al.* 2007). A strain of this bacterium harbored a new gene that exhibited activity against the Asian tiger mosquito *Aedes albopictus* (Figure 50). This mosquito species is a vector of chikungunya virus, dengue virus, and dirofilariasis, and is rapidly expanding its range due to human activity. But perhaps it is more likely that this strain and others of the species *Bacillus thuringiensis* are active against multiple pathogens, some of which do affect bryophytes. The bryophytes may also provide a service to the community by maintaining a reservoir of these bacteria that are available to the other plant species and able to render their antagonistic effects there.



Figure 50. *Aedes albopictus*, a species of mosquito that is sensitive to a bryophyte-inhabiting strain of the bacterium *Bacillus thuringiensis*. Photo by James Gathany, CDC, through public domain.

### Inducible Defenses

Bodade *et al.* (2008) provided indirect evidence that an inducible reservoir of defense compounds might be the case in the bryophytes they tested. They found that the antibacterial extracts were not always effective against the same bacterium, nor was the magnitude of inhibition consistent. This suggests the possibility of environmental stimulation by the bacteria themselves or by the environmental conditions with the possibility of seasonal changes. The interactions of bacteria with their bryophyte substrates are a new field of study with many questions needing answers.

Gimenez-Ibanez *et al.* (2019) noted that to that date no bacterial pathogens had been discovered in association with the widespread liverwort *Marchantia polymorpha* (Figure 24). In addition to this lack of evidence of bacterial pathogens, the researchers discovered an ancient immune system that governs plant-microbe interactions between *M. polymorpha* and the plant pathogenic bacterium *Pseudomonas syringae* (Figure 51). The presence of this bacterium on the liverwort activates the immune response, including effector activities inside the liverwort cells. This response also appears to be very specific and differs among the strains of *Pseudomonas syringae*.



Figure 51. *Pseudomonas syringae* on lilac leaf. This bacterium also induces *Marchantia polymorpha* to produce antibiotics. Photo by Jerzy Opiola, through Creative Commons.

Thus, it appears that at least some of the defenses are inducible. This saves energy and permits the bryophyte to maintain a larger library of defenses. Sabovljević *et al.* (2010) found that all extracts (in DMSO) from their investigated bryophytes [*Atrichum undulatum* (Figure 52), *Marchantia polymorpha* ssp. *ruderalis* (Figure 53), *Physcomitrium patens* (Figure 3)] produced antibacterial compounds against the bacteria *Escherichia coli* (Figure 19) ATCC 35210, *Pseudomonas aeruginosa* (Figure 20) ATCC 27853, *Salmonella typhimurium* (Figure 54) ATCC 13311, *Enterobacter cloacae* (human isolate; Figure 45), *Listeria monocytogenes* (Figure 55) NCTC 7973, *Bacillus cereus* (human isolate; Figure 56), *Micrococcus flavus* (Figure 57) ATCC 10240 and *Staphylococcus aureus* (Figure 18) ATCC 6538). Extracts from naturally grown bryophytes demonstrated better antibacterial activity than did those from laboratory-grown bacteria, suggesting that the presence of bacteria in the environment could stimulate production of defense compounds.



Figure 52. *Atrichum undulatum*, a moss species that produced antibacterial compounds against a number of tested bacteria. Photo by Michel Langeveld, through Creative Commons.





Figure 53. *Marchantia polymorpha* ssp. *ruderalis*, a species that produced antibacterial compounds against a number of tested bacteria. Photo by David T. Holyoak, with permission.

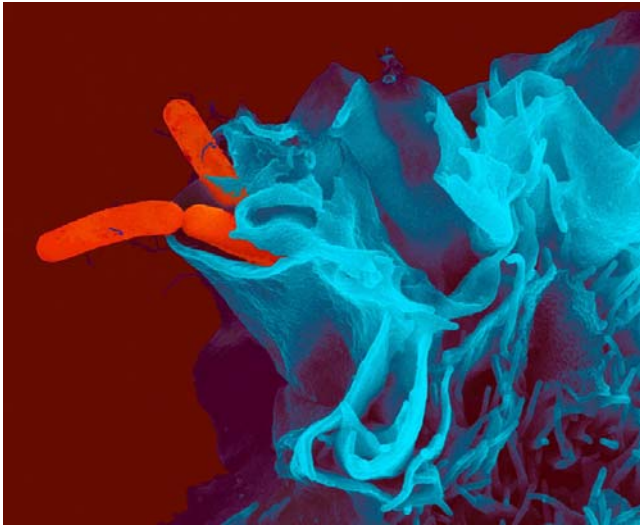


Figure 54. *Salmonella typhimurium* in human epithelial cell, a bacterial species affected by antibacterial compounds from several bryophytes. Photo by David Goulding, Wellcome Trust Sanger Institute, through Creative Commons.



Figure 55. *Listeria monocytogenes*, a bacterium inhibited by *Atrichum undulatum*, *Marchantia polymorpha* ssp. *ruderalis*, and *Physcomitrium patens*. Photo by Kateryna Kon, TheConversation.com, through Creative Commons.

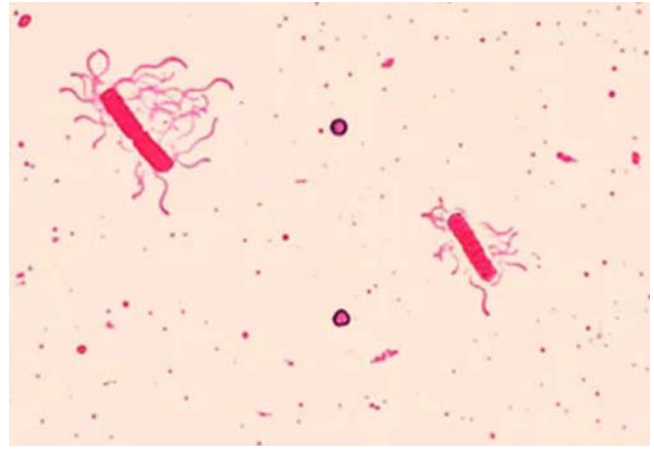


Figure 56. *Bacillus cereus*, a bacterium inhibited by *Atrichum undulatum*, *Marchantia polymorpha* ssp. *ruderalis*, and *Physcomitrium patens*. Photo by William A. Clark, CDC, through public domain

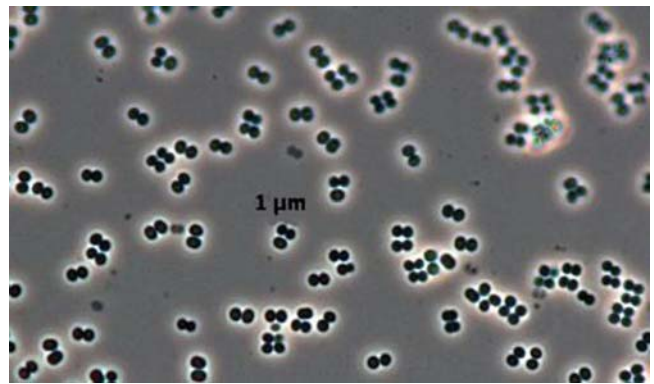


Figure 57. *Micrococcus flavus*, a bacterium inhibited by *Atrichum undulatum*, *Marchantia polymorpha* ssp. *ruderalis*, and *Physcomitrium patens*. Photo Leibniz-Institut DSMZ, through Creative Commons.

Ponce de León and Montesano (2017) noted that early bryophytes needed adaptations to combat both abiotic stresses and pathogenic microorganisms. They reported that several of the defense mechanisms against microbial pathogens were retained in the evolution of flowering plants and they provided evidence that defense compounds can, in fact, be induced. The moss *Physcomitrium patens* (Figure 3) uses plasma membrane receptor(s) to sense the pathogen. It then transduces the signal through a MAP kinase cascade that leads to activation of defenses associated with the cell wall and expression of genes encoding for proteins with various roles in plant resistance. Other responses include activation of the production of reactive oxygen species (ROS), induction of an HR-like reaction, and an increase in some hormone levels.

Alvarez *et al.* (2016) noted that the shikimate, phenylpropanoid, oxylipins, and auxin pathways were all activated by introducing the bacterium *Pectobacterium carotovorum* (Figure 29) to the moss *Physcomitrium patens* (Figure 3). The **shikimate pathway** leads to the production of phenolic compounds, which are known inhibitors of bacteria (Santos-Sánchez *et al.* 2019). **Phenylpropanoids** can work synergistically with most antibiotics and provide enhanced antibacterial activity (Hemaiswarya & Doble 2010). **Oxylipins** signal the regulation of plant growth and development, senescence,



sex determination of reproductive organs, and of importance here, the defense against biotic and abiotic stress and programmed cell death (Christensen & Kolomiets 2011). **Auxin** is a growth hormone for which concentrations, and relative concentrations, matter (Leyser 2017).

In experiments with *Physcomitrium patens* (Figure 3), Ponce de León *et al.* (2007) clearly demonstrated induction. This was achieved with the pathogenic bacterium *Pectobacterium carotovorum* ssp. *carotovorum* (Figure 29). Infection with this bacterium caused severe maceration, whereas *carotovorum* caused only mild symptoms. Both the species and subspecies induce a defense response in the moss, as evidenced by enhanced expression of conserved plant defense-related genes.

Inducible defense mechanisms in *Physcomitrium patens* (Figure 3) include reinforcement of the cell wall, production of reactive oxygen species, programmed cell death, activation of defense genes, and synthesis of secondary metabolites and defense hormones (Ponce de León & Montesano 2013). These responses are induced by the exposure to the pathogens.

All of this evidence indicates that the defense responses by the bryophytes are inductive, but it is unlikely that they are entirely inductive.

## Antioxidants and ROS

The oxidative burst is "a rapid, transient production of huge amounts of reactive oxygen species (ROS)" (Wojtaszek 1997). Changes in cell wall pH are important in controlling this production.  $H_2O_2$  (hydrogen peroxide) is produced and is directly toxic to micro-organisms (Samoilenko *et al.* 1983). The peroxide can disturb the structure and permeability of the bacterial cell wall and the cytoplasmic membrane, as well as inducing ribosomal lesions and rupturing the DNA.

In addition to being an antibacterial phenomenon, the oxidative burst is important in other plant defenses, including oxygen consumption, production of phytoalexins, systemic acquired resistance, immobilization of plant cell wall proteins, changes in membrane permeability and ion fluxes, and an apparent role in hypersensitive cell death (Wojtaszek 1997).

Unlike animal systems, plant cells are able to produce ROS, primarily as  $H_2O_2$ , in significant amounts (Wojtaszek 1997). This production is mostly exocellular and is regulated by such factors as hormones, light, and wounding. Whereas it is generally absent in elongating cells of tracheophytes, it can exhibit significant production in wounded cells or those undergoing mechanical stress. Its half-life of  $10^{-9}$  s makes it difficult to follow the sequence of reactions. In suspension cultures, pathogens such as fungi and bacteria (**elicitors**) usually elicit a response in 1-2 minutes, reaching a maximum response in several minutes (Figure 58). The reaction is completed within 30-60 minutes after initiation. Time intervals for intact plants seem to be much longer. And response time varies with the elicitor and plant species. Furthermore, the specific compound responsible for the elicitation varies among species of elicitor, as does the degree of response. But is all this true in bryophytes?

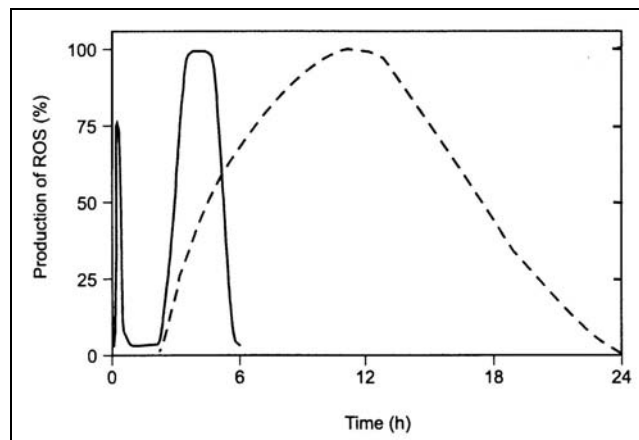


Figure 58. Oxidative burst of plant cells in response to bacterial elicitation (—) and ROS generation by plants in response to treatment with OGA (oligo-1,4- $\alpha$ -D-galacturonide) (---), a known elicitor of an oxidative burst in many plants. Modified from Wojtaszek 1997.

Minibayeva and Beckett (2001) were among the first to report details on the oxidative burst in bryophytes. They found that among the plants they tested, it was best developed in the cyanobacterial lichens, the hornwort *Anthoceros natalensis*, and two thalloid liverworts [*Dumortiera hirsuta* (Figure 40; Figure 59), *Pellia epiphylla* (Figure 60)]. The four mosses (Figure 59) and leafy liverwort in the test were almost completely unresponsive. Among the responsive species, production of  $O_2^-$  was generally higher in species from moist habitats and correlated well with plant water content at full turgor. Unfortunately, at the time of these experiments we were unaware of the importance of rate of drying on the survival success of bryophytes to dehydration. Their drying regime was extended from full hydration to an **RWC** (relative water content) of 0.05-0.10 in only 2.5 hours (Minibayeva & Beckett 2001), a time which usually prevents bryophytes from preparing for desiccation (Stark *et al.* 2013; Greenwood & Stark 2014). Nevertheless, in *Anthoceros natalensis* the rate of oxygen production was more than  $1000 \mu\text{mol g}^{-1} \text{ dry mass h}^{-1}$ , a rate 100 times that recorded for the roots of wheat (Minibayeva *et al.* 1998)!

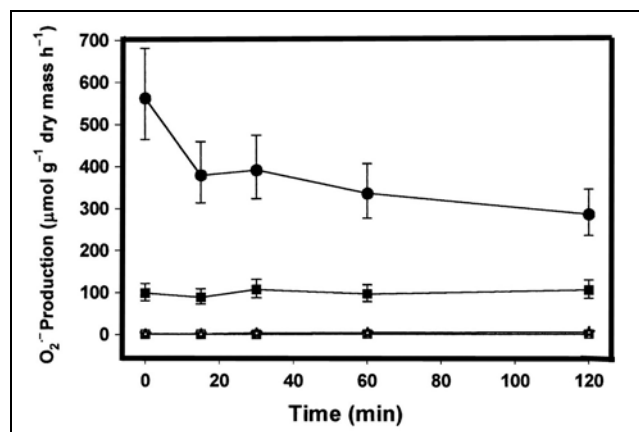


Figure 59. Superoxide production (oxidative burst) upon hydration in hydrated (solid squares) and desiccated (solid circles) *Dumortiera hirsuta* (Figure 40), and hydrated (open squares) and desiccated (star) moss *Atrichum androgynum* (Figure 61). Modified from Minibayeva & Beckett 2001.





Figure 60. *Pellia epiphylla*, a thallose liverwort that experiences a high level of oxidative burst when it is rehydrated. Photo by Bernd Haynold, through Creative Commons.

Mayaba *et al.* (2002) found that the moss *Atrichum androgynum* (Figure 61) produced an oxidative burst of hydrogen peroxide during rehydration, an ROS response. They suggested that this oxidative burst might provide protection against bacterial and fungal attempts to invade the cells. As additional support for this hypothesis, Lawton and Saidasan (2009) found that the moss *Physcomitrium patens* (Figure 3) produces reactive oxygen species (ROS) in response to pathogenic bacteria. Mayaba *et al.* found a burst of  $H_2O_2$  (oxidative burst) during rehydration during the first 15 minutes in *Atrichum androgynum*. They found that the production increased as the desiccation time of the moss increased. Light and the hormone ABA (abscisic acid) influenced the rate.



Figure 61. *Atrichum androgynum*, a species that produces an oxidative burst of hydrogen peroxide during rehydration. Photo by Nick Helme, through Creative Commons.

Lyapina *et al.* (2021) found that mosses had a higher number of small secreted peptides (SSPs) in their genomes than did either the liverwort *Marchantia polymorpha* (Figure 24) or the hornwort *Anthoceros* sp. (Figure 62). Synthetic peptide elicitors like those of tracheophytes triggered reactive oxygen species production in the protonema of the moss *Physcomitrium patens* (Figure 3), suggesting that even tracheophytes could elicit the ROS

response in the bryophytes, thus perhaps aiding in their ability to defend against invading bacteria in the protonema stage. This moss also secretes peptides that respond specifically to a chitosan treatment, indicating a possible role in immune signalling. Could these elicitors from tracheophytes be a signal to help the bryophytes determine a suitable place to become established?



Figure 62. *Anthoceros punctatus*; a tested species in this genus has fewer small secreted peptides (SSPs) in its genome than do tested mosses. Photo by Malcolm Storey, <DiscoverLife.org>, with online permission.

But reactive oxygen can be dangerous for cells because it can react in so many ways. Antioxidants can be of valuable protection to bryophytes, particularly during rehydration, scavenging the oxygen quickly before it can do too much damage (Mayaba *et al.* 2002). Seel *et al.* (1992) suggested that the antioxidants may be more important than the levels of  $H_2O_2$  in desiccation survival of bryophytes.

Vats and Alam (2013) evaluated this ROS potential in the moss *Barbula javanica*. The moss had a total phenolic content of  $30 \pm 0.96$  mg GAE/gdw. It exhibited substantial antioxidant behavior against several oxidation agents, with a reducing activity at  $1259 \pm 1.56 \mu M L^{-1}$ . Vats and Alam suggested that the high phenolic content might account for this activity. The moss *Cryphaea heteromalla* (Figure 63) similarly has a high level of protection against reactive oxygen species (ROS), which can be induced by tert-butyl hydroperoxide (Provenzano *et al.* 2019).



Figure 63. *Cryphaea heteromalla*, a moss with a high level of protection against reactive oxygen species (ROS). Photo by Hermann Schachner, through Creative Commons.



## Differences in Plant Parts

One might expect that bryophytes would protect the parts that need protection the most, thus saving energy by not producing secondary compounds where they are not needed. But which tissues are the most vulnerable for the species? Mukherjee *et al.* (2012) compared antibacterial activity in the reproductive thallus to that of the vegetative thallus of *Dumortiera hirsuta* (Figure 40). They found that the reproductive thallus showed the least antibacterial activity of the two. This appears to be an interesting aspect that needs lots more study.

## Defending Others?

Bryophytes could accomplish community service by providing antibacterial activity against pathogens that affect roots and seeds. But do they?

We do know that some bryophytes produce antibacterial substances that could protect larvae. Sevim *et al.* (2017) found that 10 [*Calliergonella cuspidata* (Figure 38), *Calliergonella lindbergii* (Figure 64), *Grimmia alpestris* (Figure 65), *Isoetecium alopecuroides* (Figure 66), *Metzgeria conjugata* (Figure 67), *Polytrichastrum formosum* (Figure 68), *Polytrichum commune* (Figure 69), *Syntrichia calcicola* (Figure 70), *Syntrichia montana* (Figure 71), *Tortella inclinata* var. *densa* (Figure 72)] out of 23 tested species of bryophytes were active against *Paenibacillus* (Figure 73) obtained from larvae of the honeybee (*Apis mellifera*). Although it is unlikely that any honeybee larvae will be living among bryophytes, other kinds of larvae do occur there and these antibiotics might protect them against bacteria as well.



Figure 64. *Calliergonella lindbergii*, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by Bob Klips, with permission.



Figure 65. *Grimmia alpestris*, on rock, with capsules, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by Henk Greven, with permission.



Figure 66. *Isoetecium alopecuroides*, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by Herman Schachner, through Creative Commons.



Figure 67. *Metzgeria conjugata*, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by Jo Denyer, with permission.





Figure 68. *Polytrichastrum formosum*, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by David T. Holyoak, with permission.



Figure 69. *Polytrichum commune*, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by Kristian Peters, through Creative Commons.



Figure 70. *Syntrichia calcicola*, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by Hermann Schachner, through Creative Commons.



Figure 71. *Syntrichia montana*, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by Claire Halpin, with permission.



Figure 72. *Tortella inclinata* var. *densa*, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by Hermann Schachner, through Creative Commons.



Figure 73. *Paenibacillus larvae* infecting a hive. Photo by Tanarus, through Creative Commons.

## Potential Uses

Bryophytes can have a number of functions in the ecosystem resulting from their providing a welcoming habitat for bacteria. For example, *Bacillus thuringiensis* (Figure 17) (*Bt*) is the source of the antibiotics in some



kinds of pesticides (Figure 74), especially against beetles, mosquitoes, black flies, caterpillars, and moths (Perez *et al.* 2015). Zhang *et al.* (2007) found that *Bacillus thuringiensis* occurs naturally on bryophytes. *Bt* is non-toxic to most animals and non-pathogenic to birds, fish, and shrimp (Perez *et al.* 2015). Some of pesticides using *Bt* are even approved for use in organic gardens. Lin *et al.* (2017) found that the bacterium *Bacillus thuringiensis* strains could be isolated from bryophyte populations in Turkey, suggesting that the bryophytes could serve as a reservoir for this important bacterium. These bacteria became established as long-term inhabitants of leaves and stems within 26 days of inoculation.



Figure 74. *Bacillus thuringiensis* damage by larvae (left) and protected by Bt genes (right). Photo from Agricultural Research Service, USDA, through public domain.

Tani *et al.* (2011) cultured bacteria in hydroponic cultures of the moss *Racomitrium japonicum* (a roof-greening moss; Figure 75) and reported that these bacteria had the potential to serve as biofertilizers for production growth of this moss species. They further found that *Methylobacterium* (Figure 76) species formed a mutualistic relationship with the moss (Tani *et al.* 2012). The moss has natural populations of methylotrophic bacteria. And the moss produces methanol. The bacteria use the methanol as a carbon source, converting methanol to CO<sub>2</sub>. When these bacteria are present in cultures of *Racomitrium japonicum*, they increase the growth of the moss – a desirable phenomenon for mosses grown in production quantities.



Figure 75. *Racomitrium japonicum*, a species that benefits from the oxidation of methanol by *Methylobacterium* and for which other associated bacteria serve as a "fertilizer" by enhancing growth. Photo by Masaki Shimamura, with permission.

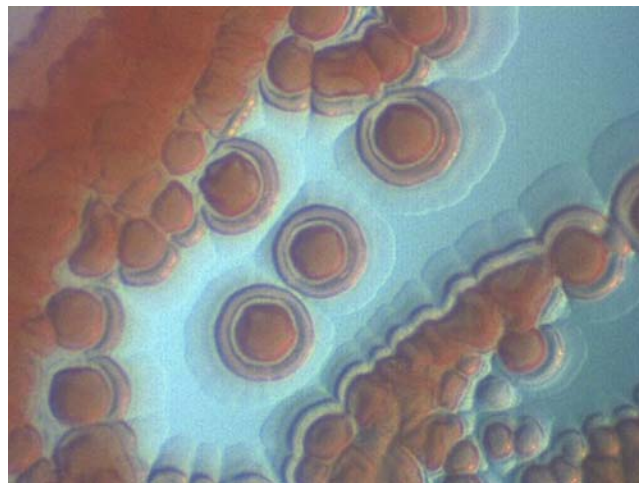


Figure 76. *Methylobacterium symbioticum*, in a bacterium genus that benefits *Racomitrium japonicum* through the oxidation of methanol. Photo by Symborg, through Creative Commons.

Mishra *et al.* (2014) remind us that many bacteria have developed resistance to most of our traditional antibiotics. They suggest the use of bryophyte antibiotic substances as potential replacements (see also Pant 1998). These bryophytes and bacteria have been living together for millions of years, perhaps longer, and the bryophyte antibiotics are still effective.

We have already seen the potential use of bryophyte compounds to inhibit multiplication of *Melissococcus plutonius* (Figure 77), one of the causal bacteria for European foulbrood disease in honeybees. Research in developing culture techniques and enhancing growth are proceeding on *Physcomitrium patens* (Figure 3) and *Marchantia polymorpha* (Figure 24) (Schwartzberg *et al.* 2004; Horn *et al.* 2021).

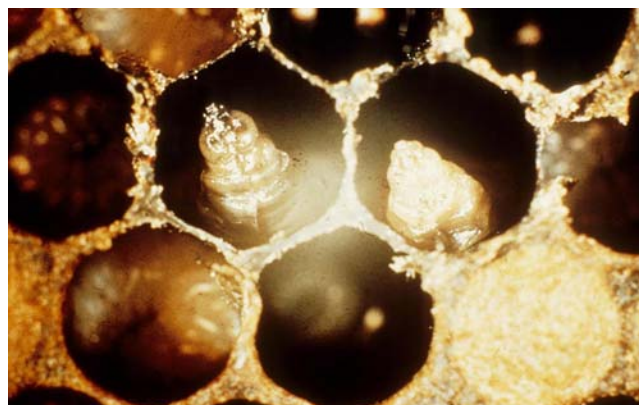


Figure 77. *Melissococcus plutonius* causing European foulbrood disease. Photo from Georgia Department of Agriculture, <Bugwood.org>, through Creative Commons.

Frahm (2004) reported that experiments at Bonn University in Germany were able to culture the first *in vivo* bryophytes for extraction of biomedical compounds. The products of all 20 tested bryophytes had effects on a variety of crop infections with various fungi. Products from bryophytes are now available commercially in Germany. In addition, successful field experiments have been completed in Peru and Bolivia. These products are



antifungal on human pathogenic fungi. But are these *in vivo* products produced by the bryophytes or by their fungal associates?

Singh *et al.* (2011) found that several bryophytes used by traditional healers were effective in the treatment of burns. The bryophyte extracts are especially effective against *Staphylococcus aureus* (Figure 18).

Mosses harbor **Actinomycetota** that include *Micromonospora chalcea* (Figure 78), a bacterium with growth promoting potential (Insuk *et al.* 2020). This species also codes for genes for phosphate solubilization, permitting the bacteria to survive in the nutrient-limited environment so common where bryophytes thrive. Their production of glycine-betaine and trehalose contribute to tolerance of drought. They have genes for heat shock proteins, cold shock proteins, and oxidative stress.



Figure 79. *Archidium ohioense*, a moss that produces substances that could provide a safer replacement for NSAIDs. Photo by Li Zhang, with permission.



Figure 78. *Micromonospora chalcea*, a bacterium that can promote plant growth and enhance drought tolerance. Photo from Leibniz-Institut DSMZ, through Creative Commons.



Figure 80. *Bryum coronatum* with capsules, a moss that produces substances that could provide a safer replacement for NSAIDs. Photo by Geoffrey Cox, through Creative Commons.

For arthritis sufferers, bryophytes have the potential to support anti-inflammatory functions. *Archidium ohioense* (Figure 79), *Bryum coronatum* (Figure 80), and *Racopilum africanum* (Figure 81) all produced substances that acted against inflammatory agents, but Ayinke *et al.* (2015) found that concentration was important. This includes protection of red blood cells effectively against heat and hypotonic induced lyses. The effects were comparable to those of expensive and somewhat dangerous non-steroidal anti-inflammatory drugs. Should we be looking for use by wild mammals for anti-inflammatory purposes, especially in the Arctic?



Figure 81. *Racopilum africanum*, a moss that produces substances that could provide a safer replacement for NSAIDs. Photo by Jan-Peter Frahm, with permission.



The potential uses of bryophytes and their associated bacteria in the pharmaceutical industry have been reviewed many times by various authors and will not be discussed further in this chapter. But it could be worthwhile to review these for their potential as a pharmaceutical chest for other animals in the wild.

## Sterilizing Bryophytes

Sterilizing bryophytes has been a challenge for bryologists. Many of the standard cleaning agents are as dangerous to the bryophytes as they are to the bacteria. Some detergents can even encourage bacterial growth (pers. obs.).

Yet it is often desirable to isolate bryophyte processes from those closely allied bacterial contributions. Hence, the decision to use sterile culture must depend on the purpose of the culture. Is it needed to understand biochemical and physiological pathways of the bryophyte, or is it needed to ascertain potential roles in the ecosystem?

This chapter has revealed that bryophytes often depend on bacteria to carry out normal life functions. On the other hand, Gupta (1977) demonstrated that the large number of bacteria associated with several bryophytes accounted for the respiratory activity measured upon rehydration of the bryophytes. They suggested that this respiration could provide an indication of survival or injury of some bryophytes, but that it presented serious limitations as indication of the cell viability of the bryophytes.

For those conditions where sterile bryophytes are needed, one must establish the conditions for growth and propagation. Schelpe (1953) tried the method of using abscised apical parts of elongated stems of mosses that have been kept in a moist atmosphere and low light intensity. Unfortunately, he had little success in obtaining bacteria-free cuttings. Lack of success in culturing bryophytes is all too common and methods differ among species.

Rowntree (2006) reported on their most successful method to date in preparing bryophytes for the Millennium Seed Bank of threatened UK bryophytes. These are held in sucrose-free  $\frac{1}{4}$  or  $\frac{1}{2}$  Murashige & Skoog or Knops minimal medium. These were successfully sterilized first (pre-cultured) with 1% (w/v) for 3 min and 0.5% (w/v) for 2 min. Sporophyte cultures were more successful than those of gametophytes due to less contamination (see also Vujičić *et al.* 2011). They found that some sterilizing treatments could cause the bryophytes to develop resistance to the toxic effects of the biocide. Vujičić *et al.* (2011) also suggested the use of sugar-free medium for *Hypnum cupressiforme* (Figure 9). They found that lower temperatures (18-20°C) also helped.

Perhaps Shaw (1986) has a better solution to culturing bryophytes while retaining the necessary interactions with bacteria, as needed for ecological studies. He has successfully cultured them by drying the bryophyte gametophytes, grinding them to a fine powder, and sowing this powder on native soil or other desired substrate. This method has the advantage of producing bryophytes with normal morphology – something that is often missing in sterile culture.

## Summary

For whatever reason, bryophytes have many secondary compounds that are antibiotic to many types of human and plant pathogens. In some cases, these are effective against bacteria that could affect the bryophytes. For both types, they are often produced only in response to the presence of certain bacteria or other microorganism. Of greater interest here are the bacteria that protect the bryophytes.

There are some implications that there are differences in quantity of antibacterial substances that depend on habitat. These differences are unclear, with some aquatic species having many such compounds and some cave thallose liverworts, a bryophyte type that usually produces high concentrations of antibacterial compounds, can have none! Part of the problem might relate to sterile culturing, or the bacteria might be unculturable species. In any case, much more must be learned before any generalizations can be made.

Among the protections exhibited by some bryophytes are oxidative bursts upon rehydration. It is suggested that this serves to protect the bryophytes at a time when their membranes are damaged and could provide easy access for the bacteria. To accompany this burst, the bryophytes can accelerate the production of antioxidants, a necessity for the bryophyte to avoid damage by free radicals of oxygen.

Little is known about differences in defense or bacterial numbers among plant parts. In some cases, reproductive parts are less protected.

Some of the bacteria produce compounds such as *Bt* that can protect honeybee larvae from disease. Our knowledge of this is very limited, but the ability of these compounds to serve as antibiotics against multiple organisms suggests that this could be a fertile area for research. The bacteria that live among bryophytes suggest that the bryophytes could serve as a reservoir of these bacteria, and that in turn the bacteria could provide antibiotics for other organisms in the ecosystem, including humans.

Bacteria can present a problem in studying the physiology of bryophytes because they contribute to the measured photosynthesis and respiration. But sterilizing the bryophyte can keep the bryophyte from developing normally or from producing substances that you are trying to measure.

It has become clear that the bacteria associated with a bryophyte can have profound effects on its success, including successful establishment, development, and growth. This is an important consideration for those attempting production levels of moss culturing.

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