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CHAPTER 19-1
BACTERIAL EFFECTS ON BRYOPHYTES

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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_2 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.

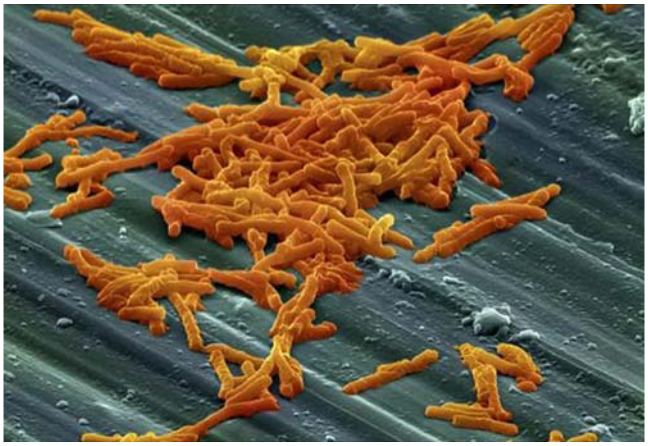

Figure 2. ***Clostridium difficile***, a predominant bacterial genus on some bryophytes. Photo through Creative Commons.

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.

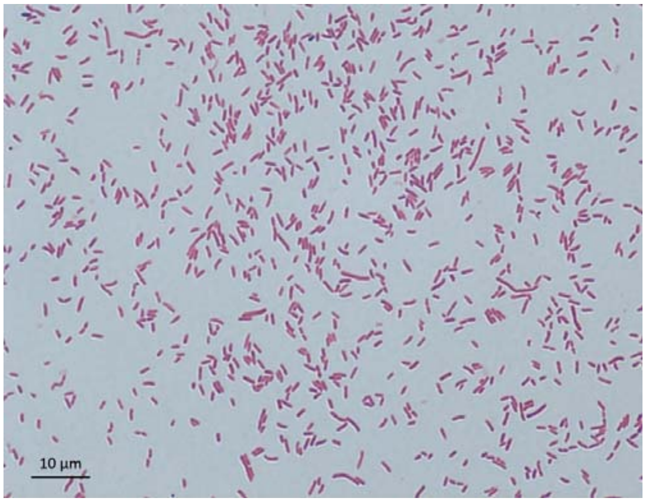

Figure 4. ***Hafnia alvei***, in one of the abundant bryophyte-dwelling bacterial genera in Japan. Photo by Antoine2003, through Creative Commons.

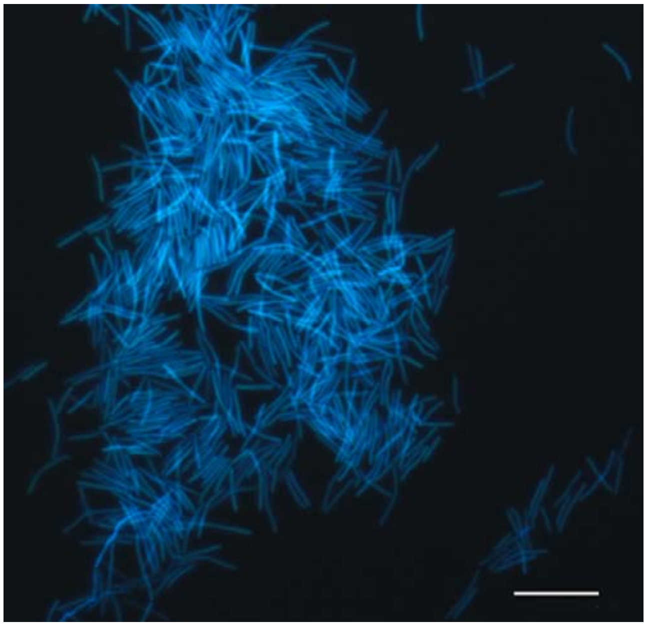

Figure 5. ***Methanobacterium*** sp., one of the abundant bryophyte-dwelling bacteria genera in Japan. Photo from JAMSTEC, through Creative Commons.

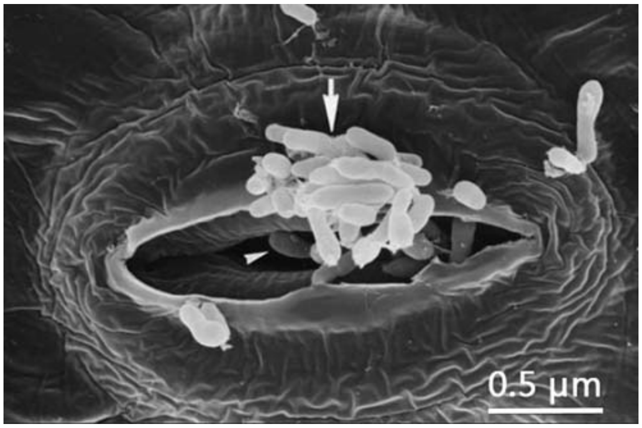

Figure 6. ***Methylobacterium*** sp. in sunflower stoma, one of the abundant bryophyte-dwelling bacterial genera in Japan. Photo by U. Kutschera, through Wikimedia Commons.

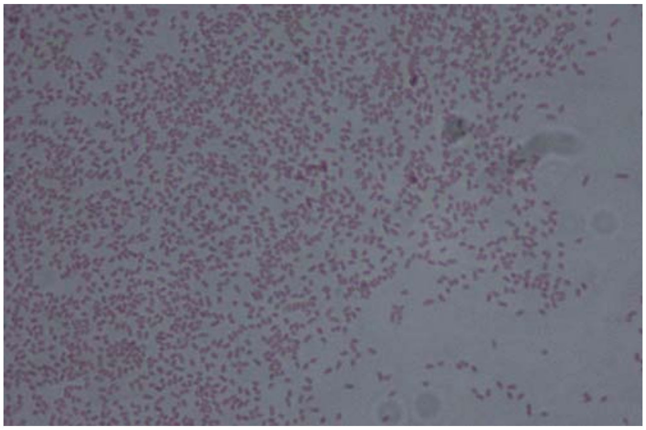

Figure 7, ***Pantoea agglomerans*** Gram stain, a species that occurs on bryophytes and is antagonistic toward some pathogenic bacteria and fungi. Photo by Dr. Sahay, through Creative Commons.

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.

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).

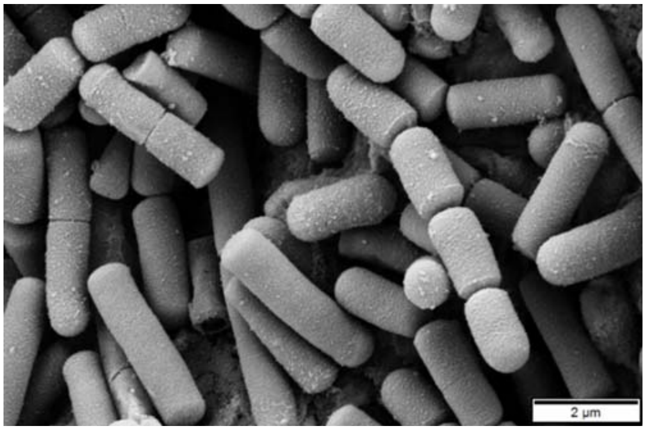

Figure 9. ***Bacillus cereus*** SEM, in an abundant genus on bryophytes in Japan and antagonistic toward them. Photo by Mogana Das Murtey and Patchamuthu Ramasamy, through Creative Commons.

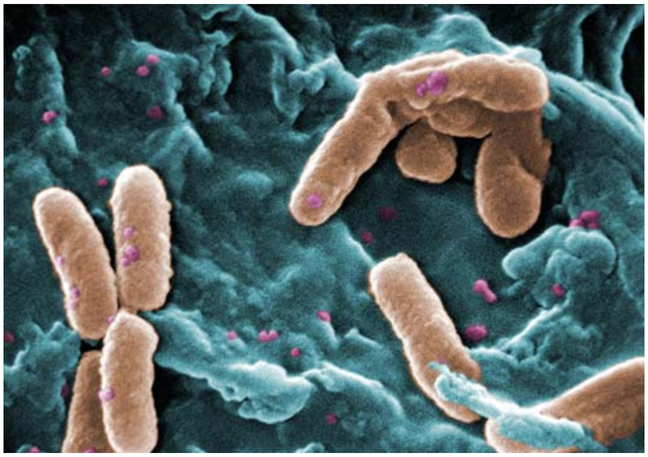

Figure 10. ***Pseudomonas aeruginosa***; ***Pseudomonas putida*** is antagonistic toward bryophytes. Photo by Janice Haney Carr, CDC, through Public domain.

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 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 **γ-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.

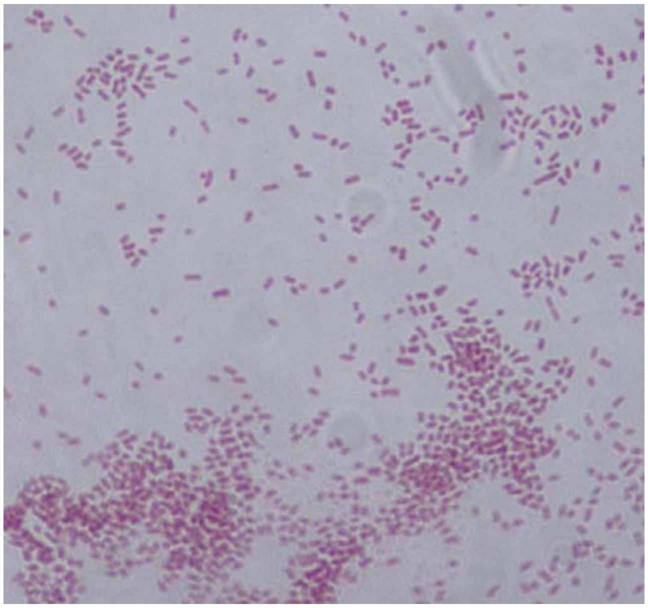

Figure 14. ***Enterobacter aerogenes***; the genus ***Enterobacter*** is a bacterial colonizer of bare-rock bryophytes in Japan. Photo by Riraq25, through Creative Commons.

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.

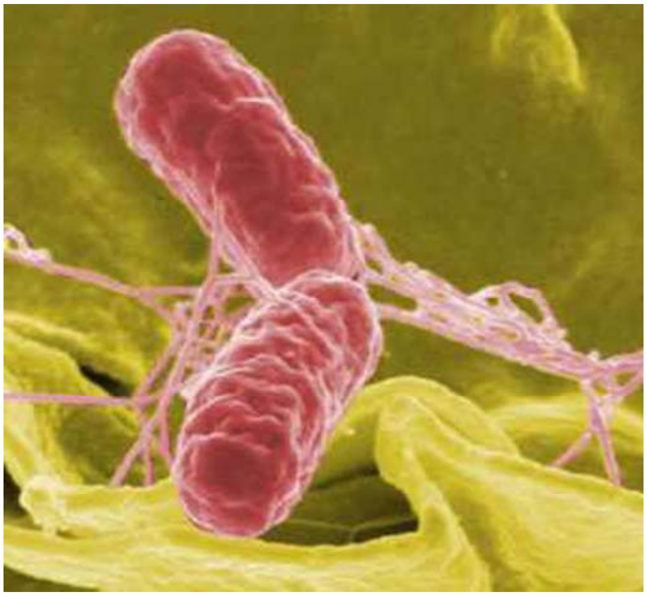

Figure 16. ***Salmonella***, bacterial colonizer of bare-rock bryophytes in Japan. Photo by JohnnyMrNinja, through Creative Commons.

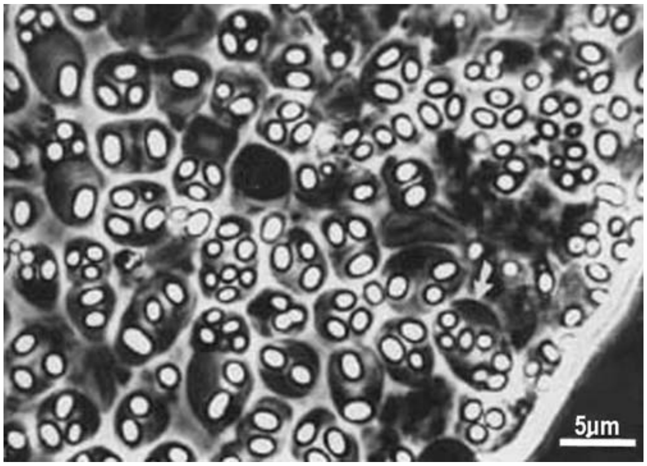

Figure 17. ***Anaerobacter polyendosporus***; members of this genus can survive extreme conditions, especially desiccation, through production of endospores. Photo by Abtop, through Creative Commons.

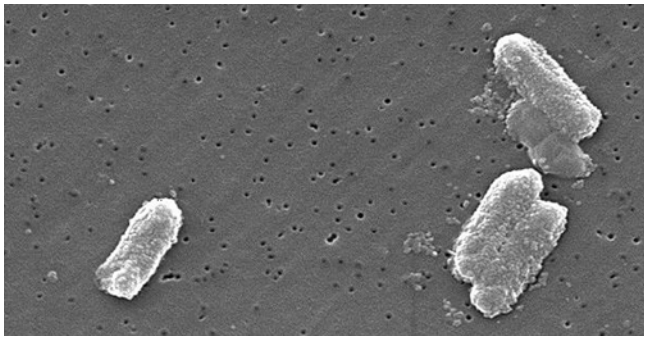

Figure 18. ***Citrobacter freundii*** SEM; some members of ***Citrobacter*** are common bacteria among highly populated soil and bare-rock-associated bryophytes. Photo through public domain.

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.

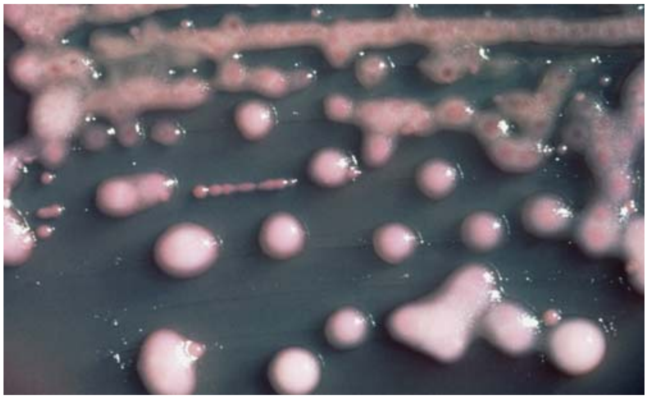

Figure 20. ***Klebsiella pneumoniae*** pink colonies; some species of ***Klebsiella*** are selective for bryophytes of highly populated soil plots. Photo from CDC, through public domain.

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.

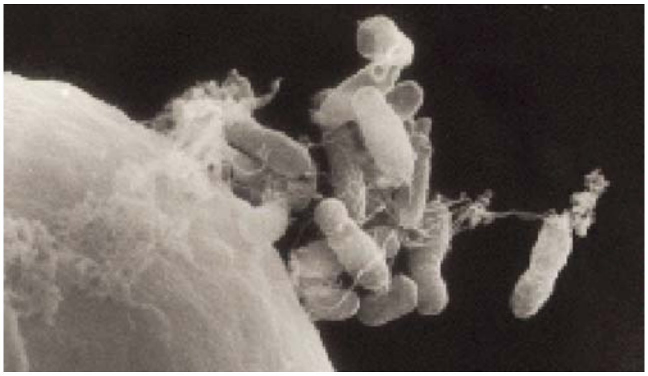

Figure 23. ***Agrobacterium tumefaciens***; an unidentified bacterium similar to ***Agrobacterium***, occurs on both surfaces of ***Polytrichum commune*** leaves. Photo through Creative Commons.

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.

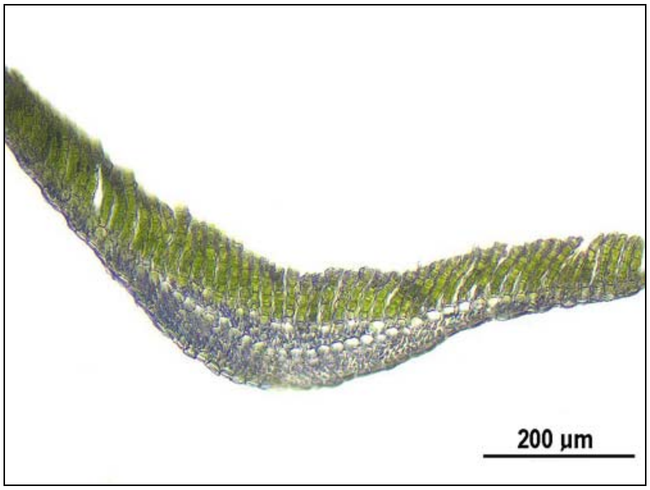

Figure 25. ***Polytrichum commune*** leaf section showing lamellae that act like an epidermis. Photo by Kristian Peters, through Creative Commons.

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), ***Frondihatitans***, ***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.

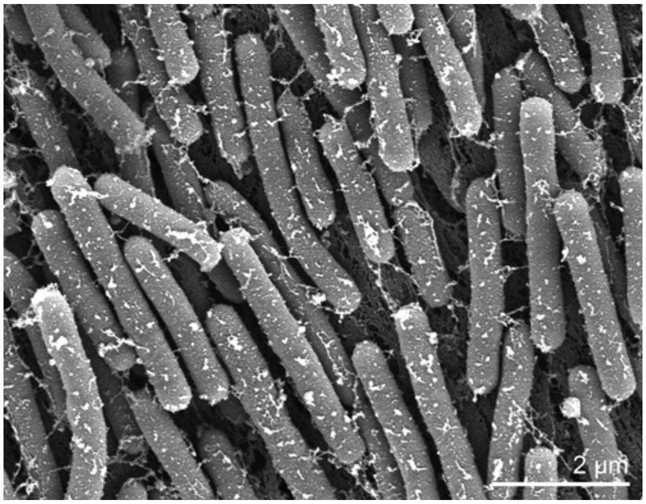

Figure 27. ***Haliangium ochraceum***, in one of the most common genera of bacteria among bryophytes in Tibet. Photo by Manfred Rohde, through Creative Commons.

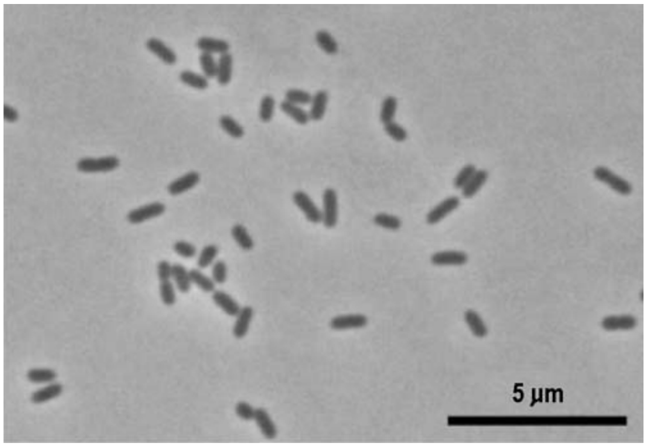

Figure 28. ***Novosphingobium***, one of the most constant genera among bryophytes in Tibet. Photo by Nierychlo *et al.*, through Creative Commons.

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 (1*n* 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 ***Anoectangium 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 **γ-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. ***Anoectangium compactum***; ***Anoectangium 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.

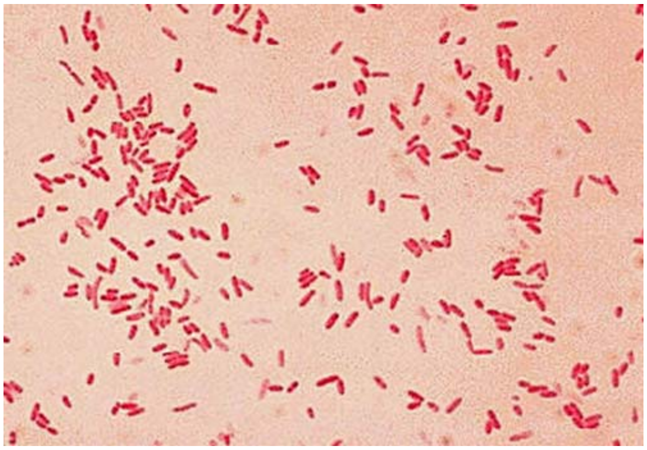

Figure 35. ***Aeromonas hydrophila***, in one of most common bacterial genera on bryophytes in the various habitats of soil, near water, and on rocks in Japan. Photo by W. A. Clark, CDC, through public domain.

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.

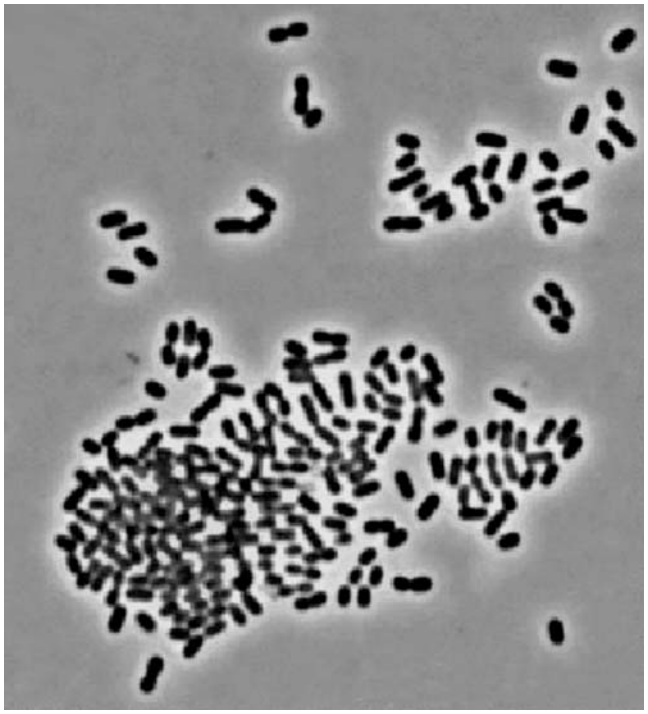

Figure 39. ***Bryobacter aggregatus***, in a genus that contributes to plant growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression in species of ***Marchantia***. Photo courtesy of the U.S. National Library of Medicine.

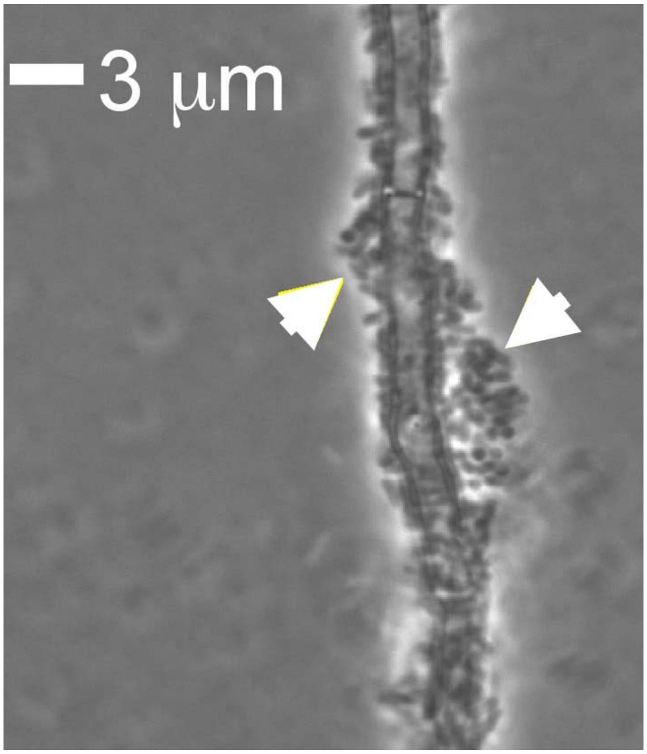

Figure 40. ***Lysobacter***, a genus that contributes to plant-growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression in species of ***Marchantia***. Photo through Creative Commons.

Figure 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.

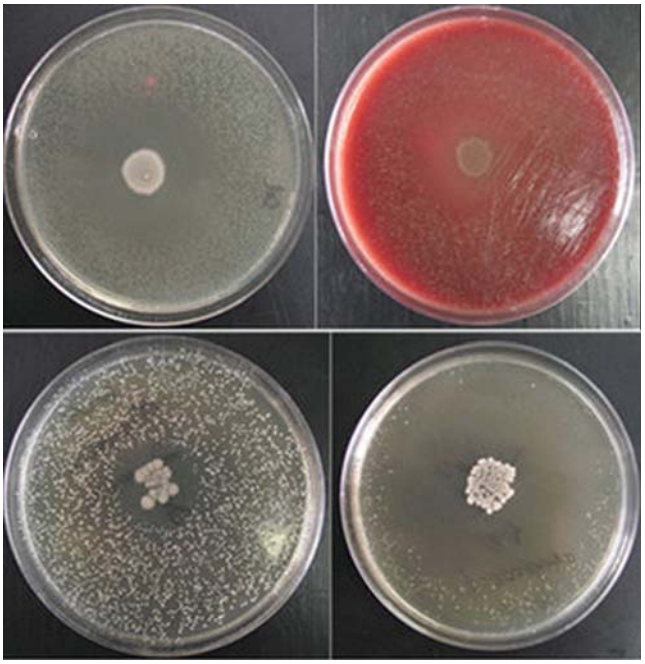

Figure 43. ***Steroidobacter denitrificans*** growth inhibition zones on various media; members of this genus contribute to plant growth promotion, complex exudate degradation, nitrogen fixation, methanol conversion, and disease suppression in species of ***Marchantia***. Photo through Creative Commons.

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.

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

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.

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 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.

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 co-polyesters. 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.

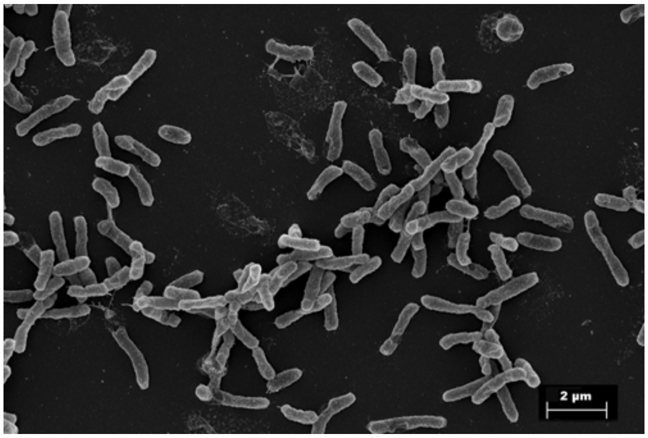

Figure 51. ***Rheinheimera baltica*** SEM; some members of this genus grow in association with ***Grimmia montana*** on bare rocks, where they are suspected of enhancing the moss growth by inhibiting other microbes. Photo by Manfred Rohde, through Creative Commons.

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 protocooperation, 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.

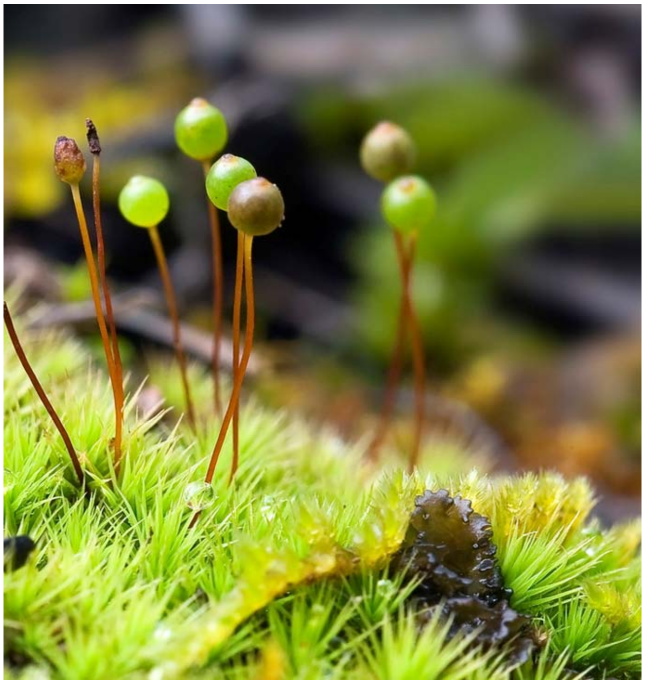

Figure 55. ***Bartramia stricta*** with capsules; a species of ***Methylobacterium*** in Spain uses methanol as its only source of carbon and energy. This is emitted by the moss and provides needed carbon for the ***Methylobacterium***, which in turn releases CO_2 used by the moss. Photo by John Game, 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***.

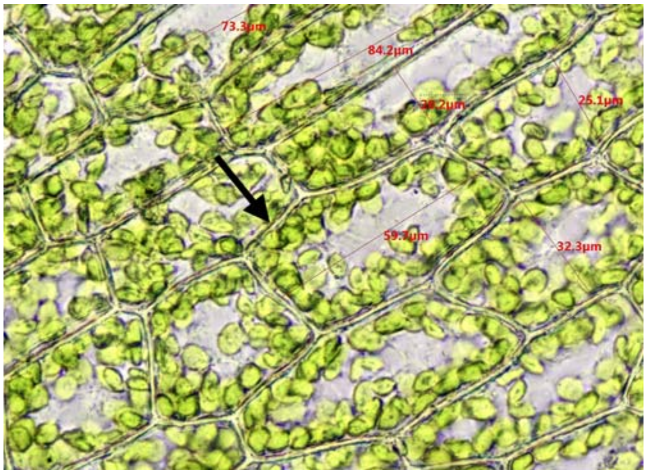

Figure 58. ***Funaria hygrometrica*** leaf cells; **arrow** indicates groove between two adjacent lamina cells where bacteria often grow. Photo by Claire Halpin, with permission.

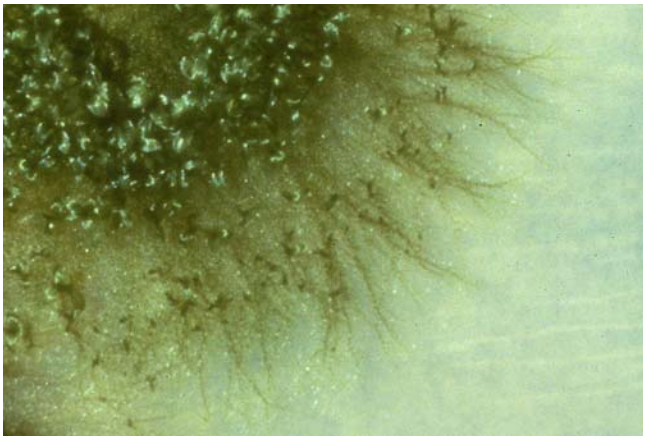

Figure 59. ***Funaria hygrometrica*** cultures with young gametophores and gametophore buds near the ends of the protonemata. ***Methylobacterium*** elicits a cytokinin type of response in the growth and bud formation of the protonema. Photo by Janice Glime.

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_2 Source

One of the first considerations regarding bryophyte interactions with bacteria was that bacteria provide a source of CO_2 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_2 even at low *p*H 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_2 becomes available in the rhizosphere sediments, the reliance on CO_2 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_2 concentrations within these two communities differed "substantially." At the beginning of the growing season, both communities had the same CO_2 concentrations of about 350 ppm. But in the turf, the CO_2 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_2 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_2 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 5ºC. 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_2. This situation illustrates the ability of high CO_2 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_2 that is available to the aquatic bryophytes.

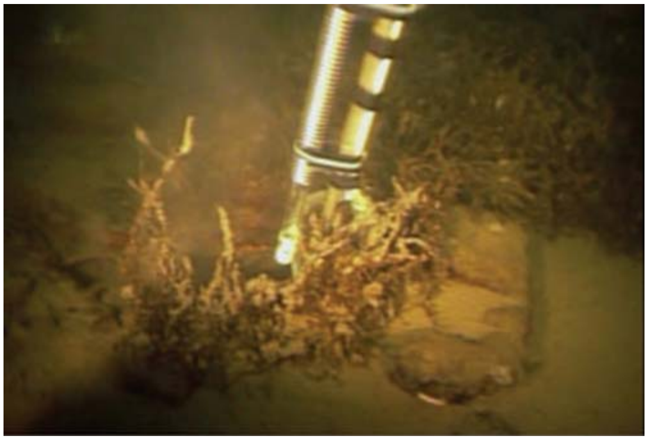

Figure 61. ***Fontinalis** cf. **novae-angliae*** from Yellowstone Lake geothermal vent, where bacterial respiration most likely contributes to its needed CO_2. Photo from Lovalvo *et al.* 2010.

Figure 62. ***Fontinalis novae-angliae*** habitat in a stream; bacteria associated with the moss most likely contribute CO_2 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_2.

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 protocooperative 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_{12}, activated charcoal, coconut milk, and altered hydration, *p*H, 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 Luretta 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.

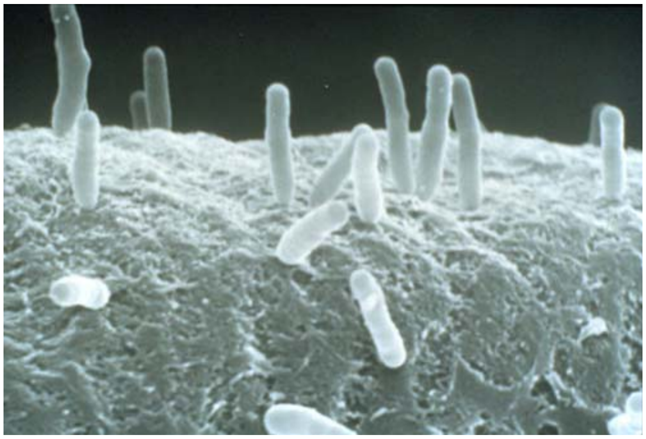

Figure 68. ***Agrobacterium tumefaciens***, a species that provides hormones needed for the development of ***Pylaisiella selwynii***. Photo by Martha Hawes, University of Arizona through NSF public domain.

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. γ-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.

Whatley 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 β-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, *p*H, 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 it 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.

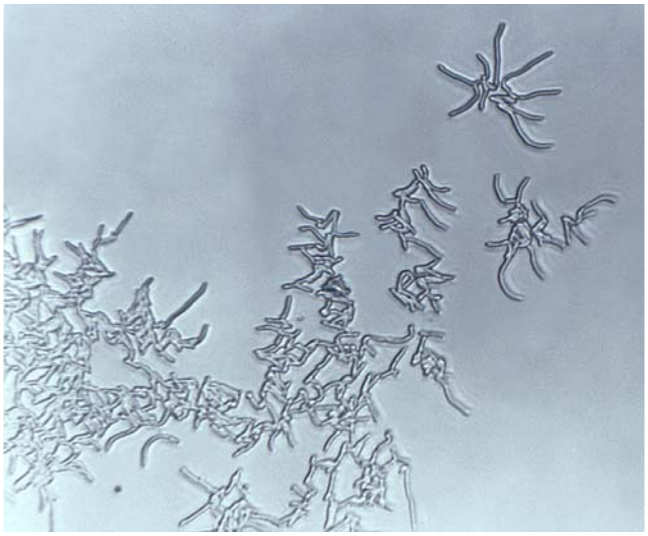

Figure 76. ***Rhodococcus***, a bacterium that enhances growth and production of ***Racomitrium japonicum***. Photo by David Berd, CDC, through public domain.

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 (**AHL**s) (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 µM. 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.

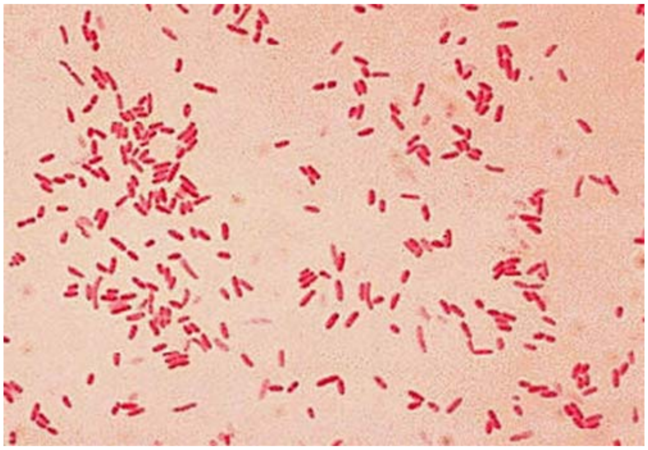

Figure 77. ***Aeromonas hydrophila***, a bacterium that produces AHLs as signalling compounds. Photo by W. A. Clark, CDC, through public domain.

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_{12}** that is manufactured by bacteria, a symbiotic need generated by the lack of B_{12}-dependent enzymes in algae (Croft *et al.* 2005). Bryophytes likewise obtain vitamin B_{12} 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 ***Liochlaena lanceolata*** (Figure 78) and ***Gymnocolea inflata*** (Figure 79) was significantly stimulated by the pink facultative methylotrophic bacteria that both synthesize and accumulate vitamin B_{12}. These bacteria commonly associate with bryophytes, but the physiological role of vitamin B_{12} is elusive (Marsten 1952).

Figure 78. ***Liochlaena lanceolata***, a species that is stimulated by the pink facultative methylotrophic bacteria that both synthesize and accumulate Vitamin B_{12}. 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_{12}. 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. ***Pellia 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_2 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 0ºC, but requires the temperature to drop to -38ºC 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 0ºC. 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^{-1}, 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 -7ºC when the spores were contaminated with bacteria, compared to -12ºC 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.

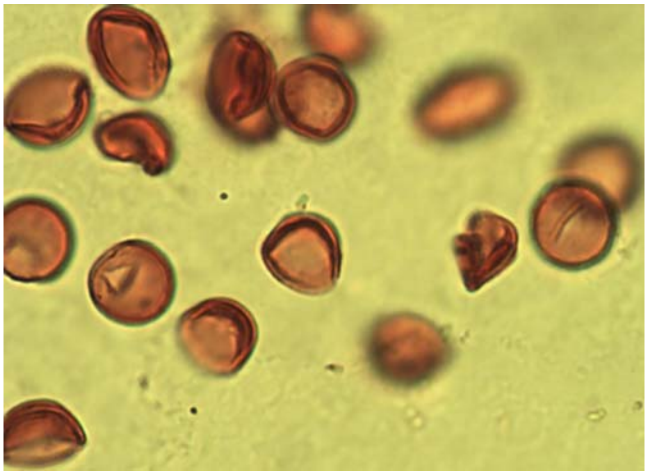

Figure 88. ***Polytrichum commune*** spores; these serve as ice nucleators in the atmosphere and are active at even higher temperatures when they have bacteria. Photo by Global Pollen Project, through Creative Commons.

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 NH_4^+ and increasing concentrations of 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 NH_4^+ and increasing concentrations of 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.

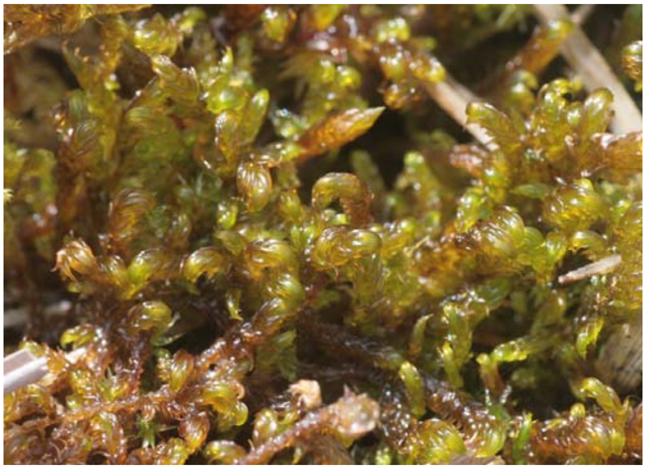

Figure 90. ***Drepanocladus cossonii***, a species limited primarily by decreasing concentrations of NH_4^+ and increasing concentrations of NO_3^+. Seasonal variation in this bryophyte cover resulted from synergism with nitrifying bacteria and by unstable water table. Photo by Hermann Schachner, through 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 *p*H. 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 *p*H 4.5-7.2 and 4-33ºC.

Again in 2014 Kulichevskaya *et al.* described a new species, genus, and family of bacteria from ***Sphagnum***. The species, ***Roseiarcus fermentans***, is a microaerophilic fermentive 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 flexuosum* (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 bactivorous 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.

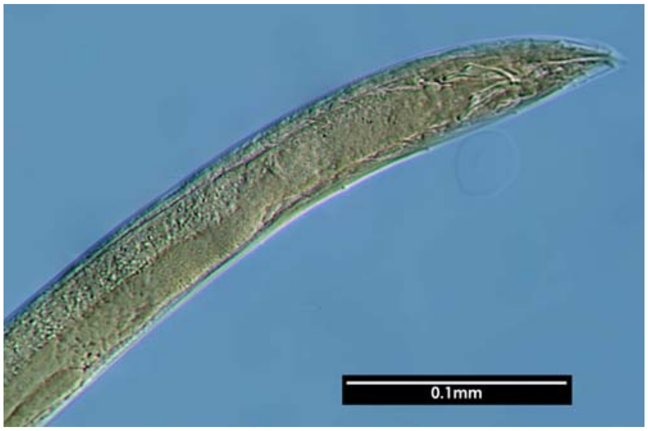

Figure 100. ***Panagrolaimus davidi***, in a nematode genus that has bacterial feeders that live among bryophytes. Photo from Smithsonian, through Creative Commons.

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.

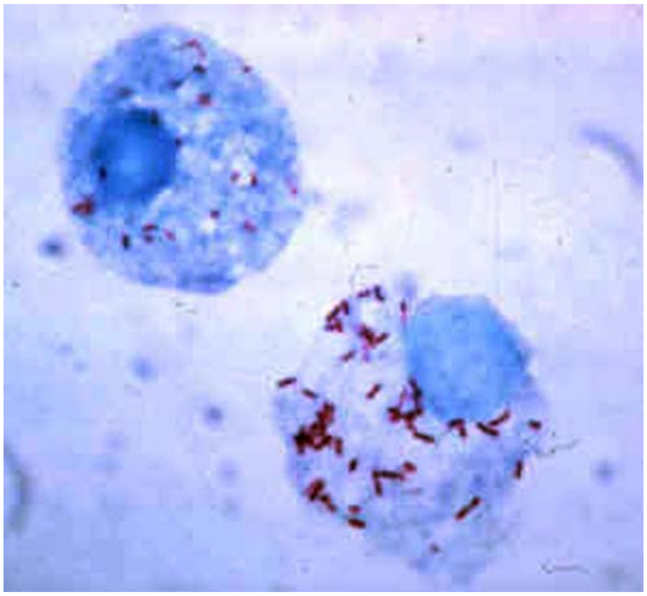

Figure 104. ***Rickettsia rickettsii***, a bacterium found in the Malpighian tubules of the moss bugs (**Peloridiidae**). Photo from CDC, through Creative Commons.

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.

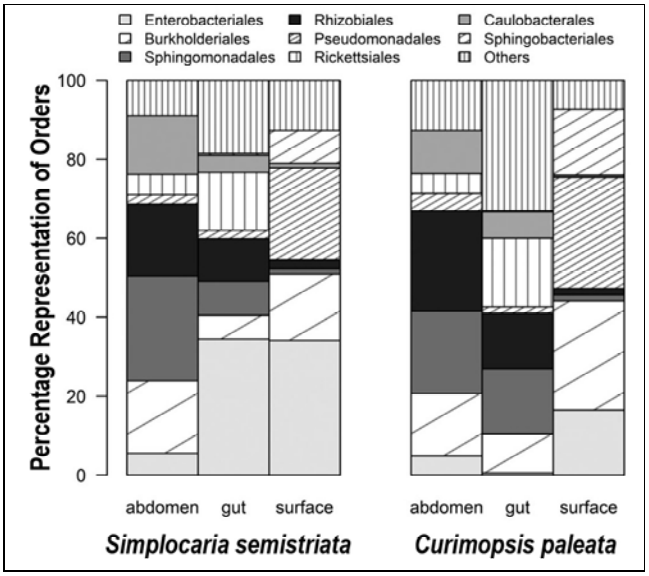

Figure 107. Composition of orders of bacteria in communities associated with abdomens, guts, and surfaces of the bryophyte-eating beetles ***Simplocaria semistriata*** and ***Curimopsis paleata***. Modified from Pyszko *et al*. 2019.

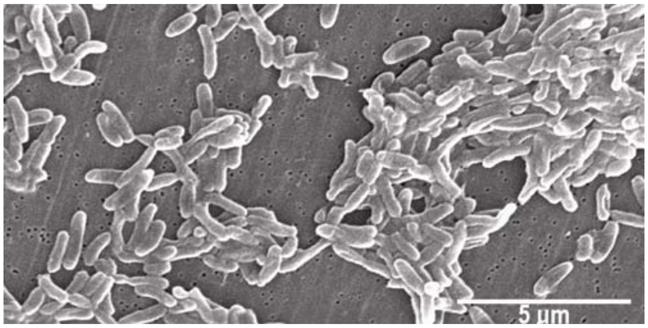

Figure 108. ***Ralstonia mannitolilytica***, a bacterium involved in nitrogen fixation or detoxification of secondary metabolites and that occurs in the guts of the bryophage beetles ***Curimopsis paleata*** and ***Simplocaria semistriata***. Photo by Judith Noble-Wang, CDC, through public domain.

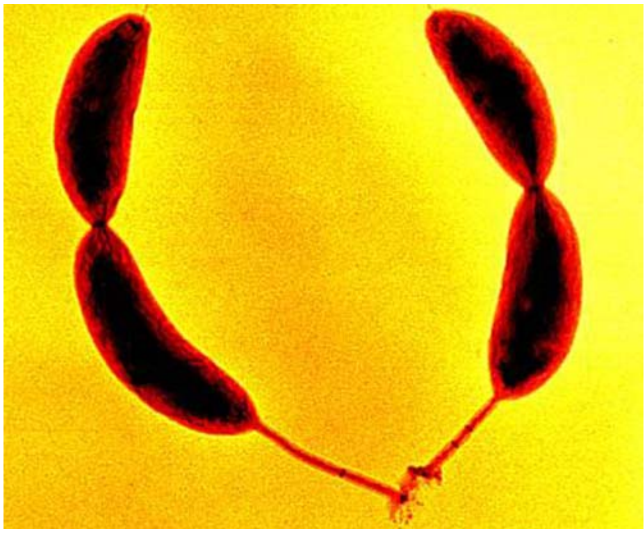

Figure 109. ***Caulobacter crescentus***, a bacterium involved in detoxification of secondary metabolites or nitrogen fixation and that occurs in the guts of the bryophage beetles ***Curimopsis paleata*** and ***Simplocaria semistriata***. Photo from USDA, through public domain.

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.

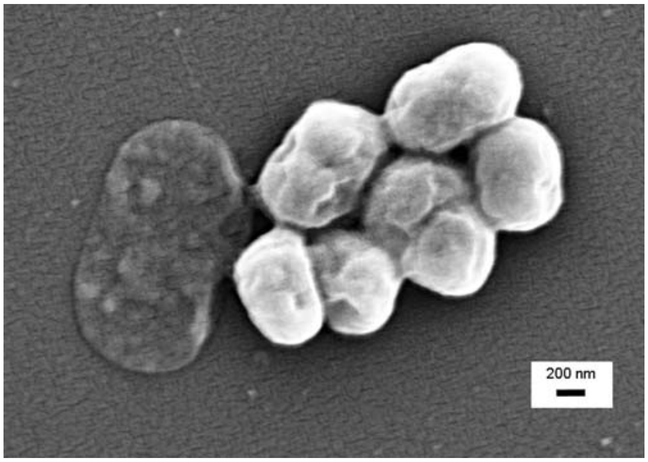

Figure 112. ***Acinetobacter baumannii*** SEM, in a genus that occurs in the alimentary canals of two oribatid mite genera. Photo by Vader1941, through Creative Commons.

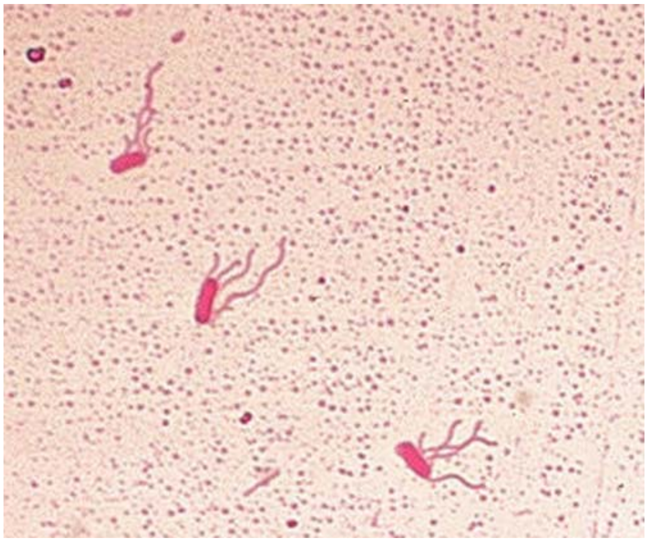

Figure 113. ***Alcaligenes faecalis***, in a genus that occurs in the alimentary canals of two oribatid mite genera. Photo by W.A. Clark, CDC, through public domain.

Figure 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 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 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 Buiobuione, 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).

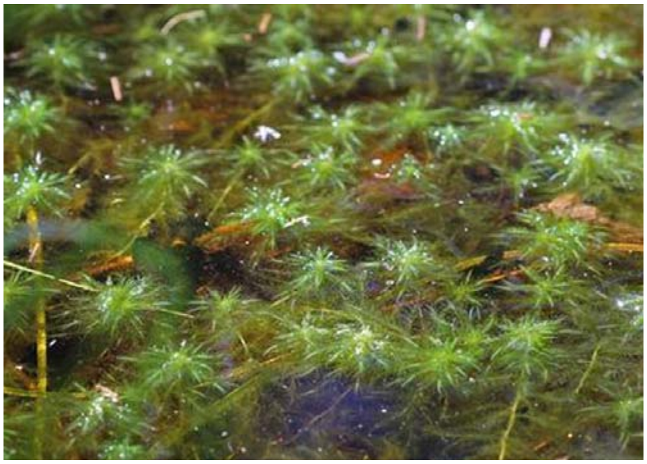

Figure 121. ***Sphagnum cuspidatum***, a habitat where ***Bacillus*** has a high frequency. Photo by Rob Routledge, through Creative Commons.

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.

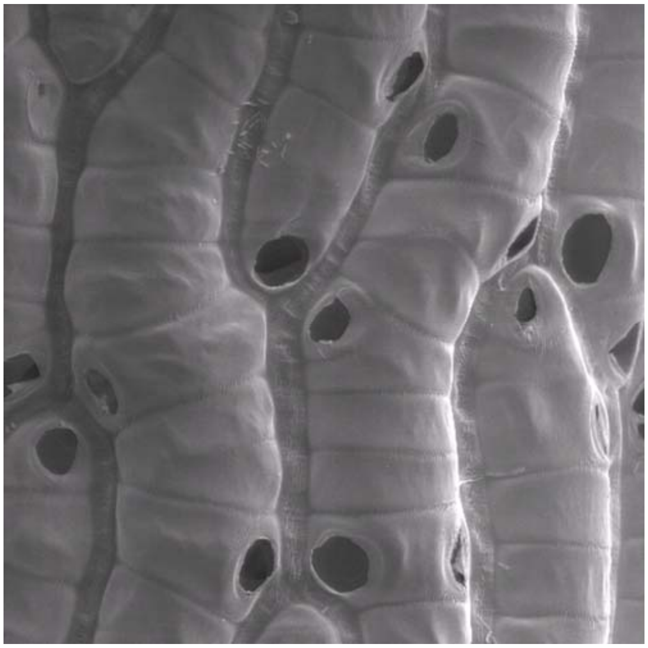

Figure 124. ***Sphagnum fallax*** hyaline cells with bacteria. Photo by Gabi Berg, with permission.

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_2 (then available to bryophytes for photosynthesis) and to fix atmospheric nitrogen (also used by bryophytes). This implies that theses bacteria somehow provide anaerobic conditions within the cells to permit nitrogen fixation to occur.

At least some, perhaps all, bryophytes obtain vitamin B_{12} 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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