Glime, J. M. 2022. Bryophyte Defenses against Bacteria. Chapt. 19-3. In: Glime, J. M. Bryophyte Ecology. Volume 2.
Interactions. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Last updated
27 March 2022 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology/>.

19-3-1

CHAPTER 19-3
BRYOPHYTE DEFENSES AGAINST BACTERIA

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

Defenses Against Bacteria

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

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

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

2012). Bryophytes also produce polyunsaturated C_{20} fatty acids. These include arachidonic acid and eicosapentaenoic acid (Ponce de León *et al*. 2015). These can be oxidized and transformed into bioactive compounds.

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

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

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

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

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

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

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

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

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

Figure 5. *Cylindrocolea recurvifolia*, a leafy liverwort with small and few oil bodies and less antibiotic activity than that of *Pleurozia subinflata*. Photo from Museum of Hiroshima University, with permission.
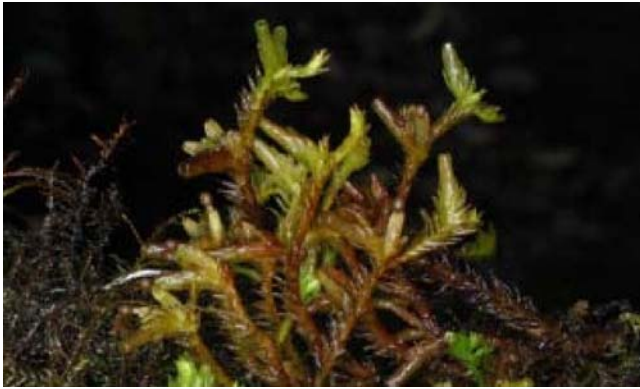
Figure 6. *Pleurozia subinflata*, a leafy liverwort with larger and more numerous oil bodies than those of *Cylindrocolea recurvifolia* and with greater antibiotic activity. Photo by Jan-Peter Frahm, with permission.

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

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

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

Antibiotic Response by Bryophytes

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

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

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

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

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

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

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

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

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

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

Figure 15. *Bacillus megaterium*, one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo by Alexastely, through Creative Commons.
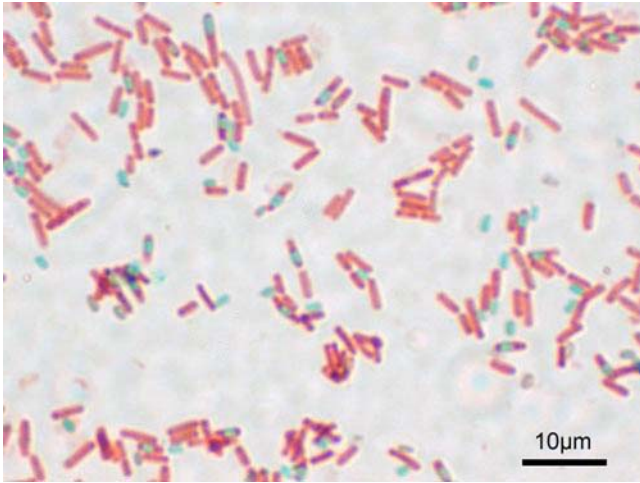
Figure 16. *Bacillus subtilis* forming spores, one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo by Y. Tambe, through Creative Commons.
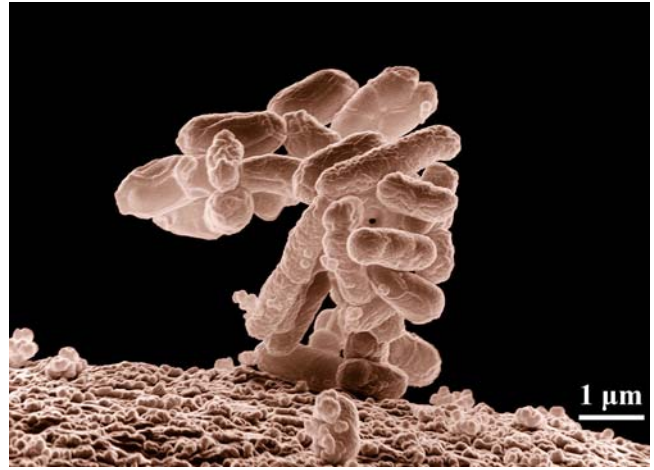

Figure 19. *Escherichia coli*, one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo by Erbe, digital colorization by Christopher Pooley, both of USDA, ARS, EMU, through public domain.

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

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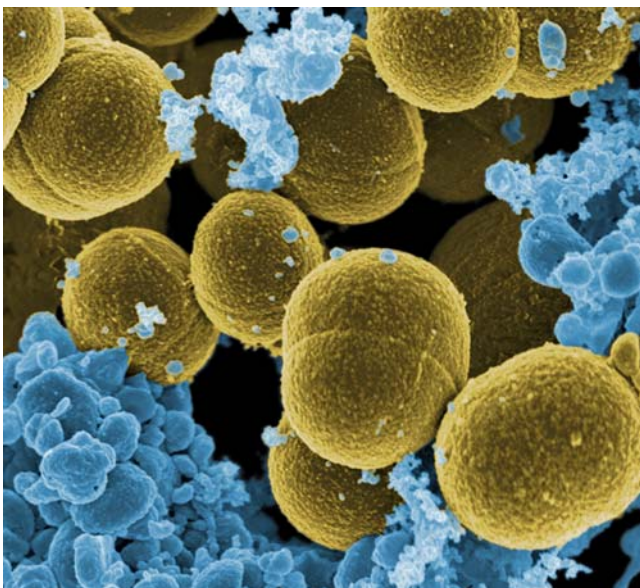

Figure 18. *Staphylococcus aureus*, one of seven bacterial species inhibited by 17 of 60 bryophyte species tested. Photo from NIAID-RML, through public domain.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Habitat Differences?

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

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

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

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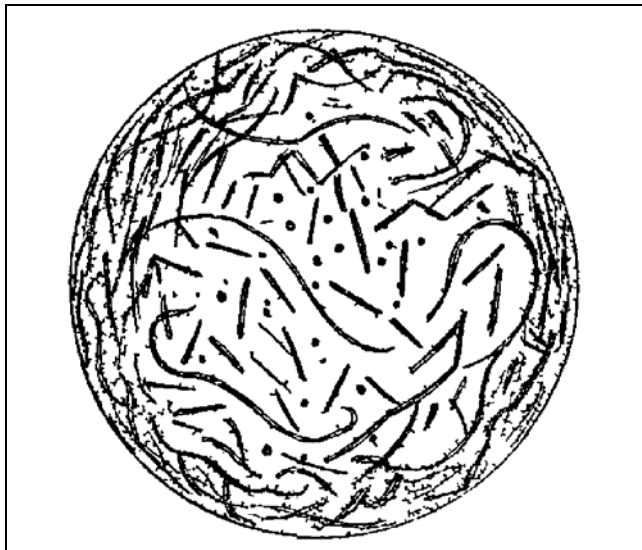

Figure 33. *Proteus vulgaris* drawing, the species that suffered the least effect by *Ditrichum pallidum* extracts in bests by Liu and Wang (2010). Image from Project Gutenberg Distributed Proofreaders, Encyclopædia Britannica, 1911, through public domain.

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

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

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

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

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

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

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

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

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

Dey *et al.* (2015) found that elevation made a difference in antibacterial activity of *Pellia endiviifolia* (Figure 39) from the eastern Himalayas. Those collected at higher elevations had significantly higher antimicrobial activity. They suggested this might be due to differences in UV light levels, with the intensity increasing at higher elevations.

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

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

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

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

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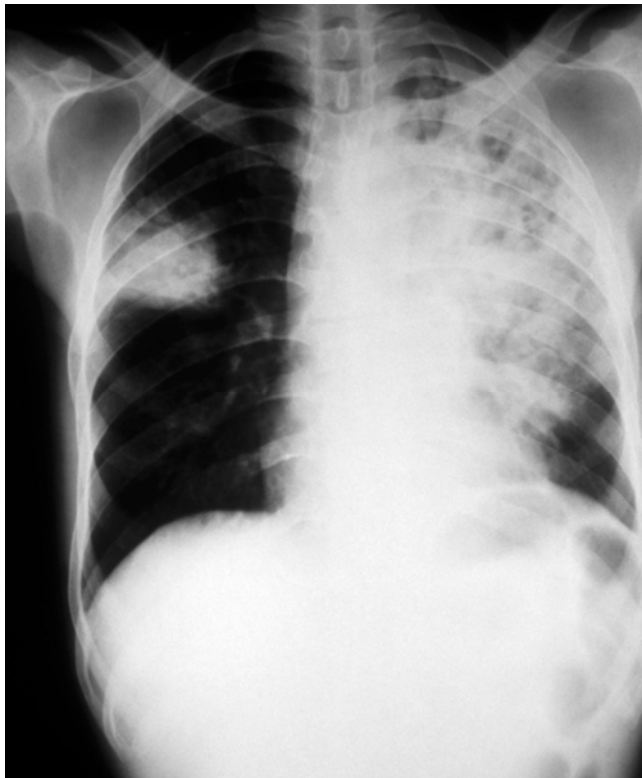

Figure 42. Human lung X-ray showing damage by ***Klebsiella pneumoniae***, especially in left lung; ***Pogonatum marginatum*** fails to respond to this bacterium. Photo through Creative Commons.

Bacterial Defense Partners

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

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

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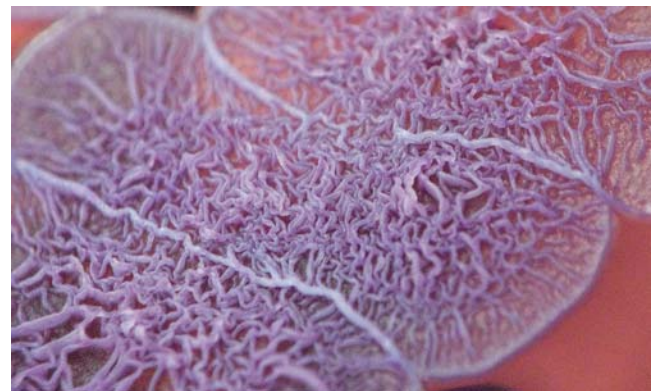

Figure 44. ***Burkholderia thailandensis***; members of this genus control proliferation and facilitate competitive exclusion of plant pathogens that occur on bryophytes. Photo through Creative Commons.

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

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

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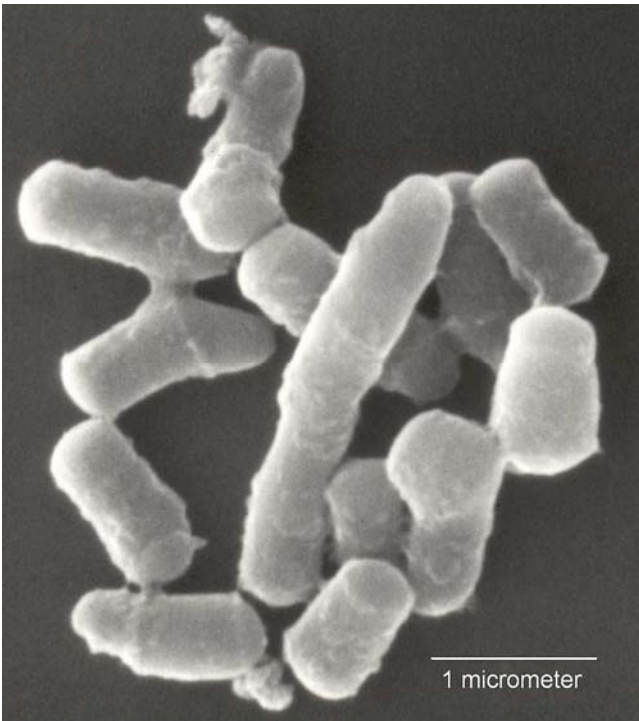

Figure 46. *Rhodococcus*; members of this genus control proliferation and facilitate competitive exclusion of plant pathogens that occur on bryophytes. Photo by Jerry Sims, through public domain.

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

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

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

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

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

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

Inducible Defenses

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

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

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

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

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

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

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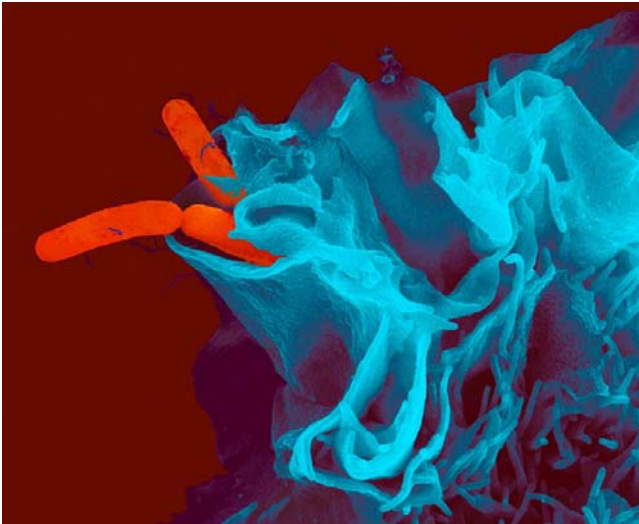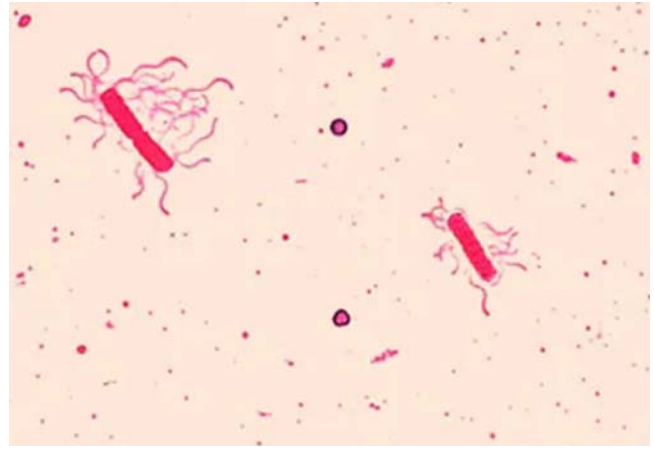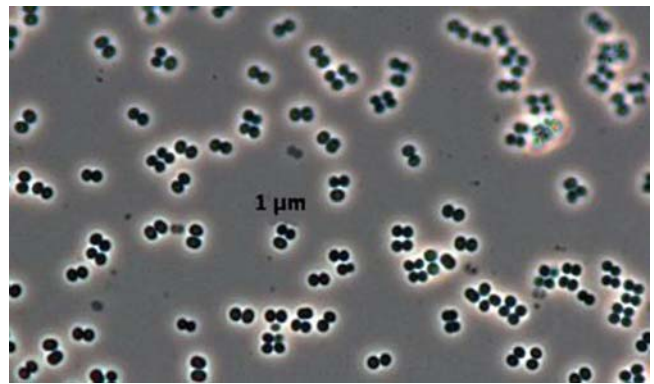

Figure 54. *Salmonella typhimurium* in human epithelial cell, a bacterial species affected by antibacterial compounds from several bryophytes. Photo by David Goulding, Wellcome Trust Sanger Institute, through Creative Commons.

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

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

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

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

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

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

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

Antioxidants and ROS

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

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

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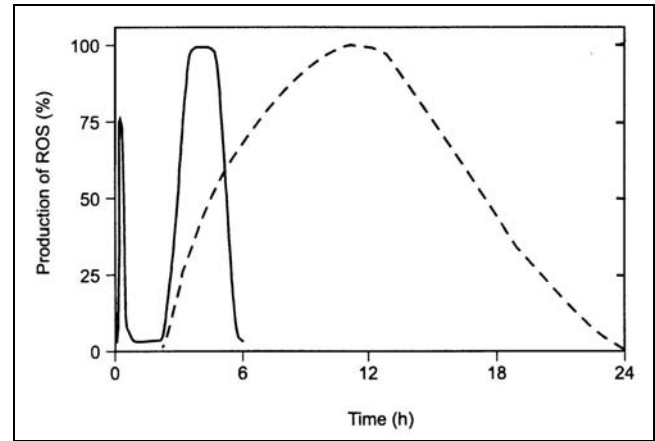

Figure 58. Oxidative burst of plant cells in response to bacterial elicitation (—) and ROS generation by plants in response to treatment with OGA (oligo-1,4-α-D-galacturonide) (– –), a known elicitor of an oxidative burst in many plants. Modified from Wojtaszek 1997.

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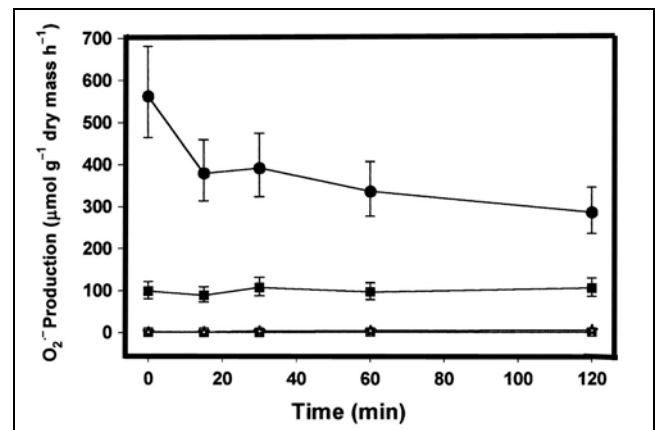

Figure 59. Superoxide production (oxidative burst) upon hydration in hydrated (**solid squares**) and desiccated (**solid circles**) *Dumortiera hirsuta* (Figure 40), and hydrated (**open squares**) and desiccated (**star**) moss *Atrichum androgynum* (Figure 61). Modified from Minibayeva & Beckett 2001.

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

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

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

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

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

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

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

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

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

Differences in Plant Parts

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

Defending Others?

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

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

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

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

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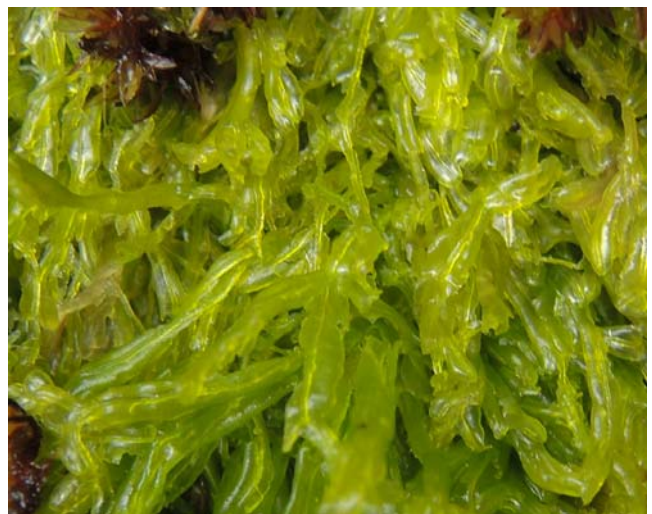

Figure 67. *Metzgeria conjugata*, a species that is active against *Paenibacillus* obtained from larvae of the honeybee. Photo by Jo Denyer, with permission.

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

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

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

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

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

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

Potential Uses

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

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

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

Tani *et al*. (2011) cultured bacteria in hydroponic cultures of the moss **Racomitrium japonicum** (a roof-greening moss; Figure 75) and reported that these bacteria had the potential to serve as biofertilizers for production growth of this moss species. They further found that **Methylobacterium** (Figure 76) species formed a mutualistic relationship with the moss (Tani *et al*. 2012). The moss has natural populations of methylotrophic bacteria. And the moss produces methanol. The bacteria use the methanol as a carbon source, converting methanol to CO_2. When these bacteria are present in cultures of **Racomitrium japonicum**, they increase the growth of the moss – a desirable phenomenon for mosses grown in production quantities.

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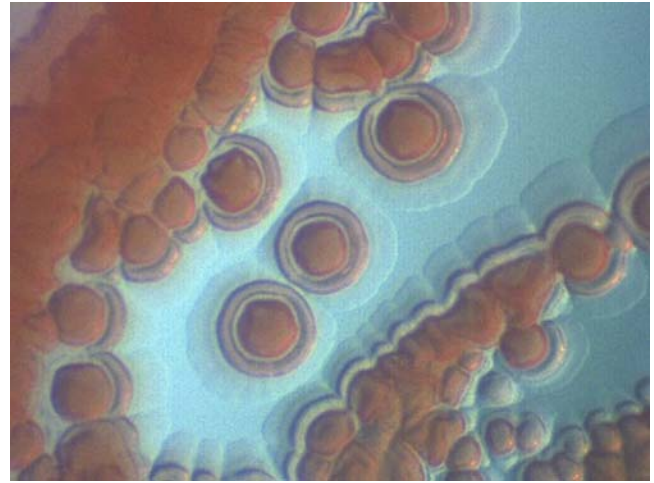

Figure 76. **Methylobacterium symbioticum**, in a bacterium genus that benefits **Racomitrium japonicum** through the oxidation of methanol. Photo by Symborg, through Creative Commons.

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

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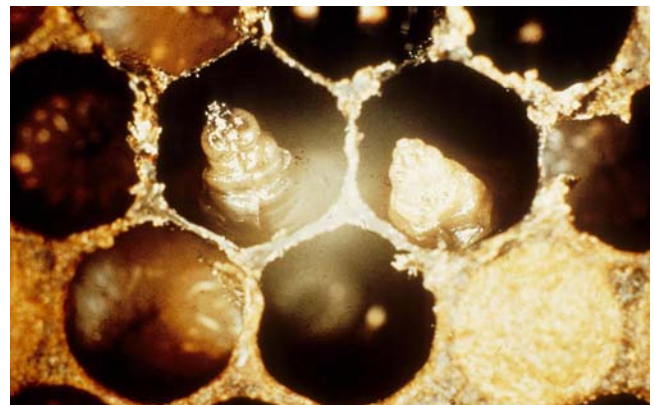

Figure 77. **Melissococcus plutonius** causing European foulbrood disease. Photo from Georgia Department of Agriculture, <Bugwood.org>, through Creative Commons.

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

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

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

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

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

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

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

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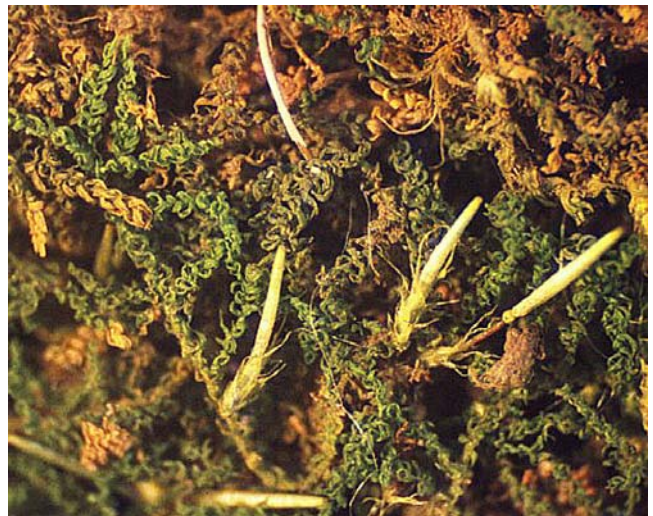

Figure 81. *Racopilum africanum*, a moss that produces substances that could provide a safer replacement for NSAIDs. Photo by Jan-Peter Frahm, with permission.

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

Sterilizing Bryophytes

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

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

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

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

Rowntree (2006) reported on their most successful method to date in preparing bryophytes for the Millennium Seed Bank of threatened UK bryophytes. These are held in sucrose-free ¼ or ½ Murashige & Skoog or Knops minimal medium. These were successfully sterilized first (pre-cultured) with 1% (w/v) for 3 min and 0.5% (w/v) for 2 min. Sporophyte cultures were more successful than those of gametophytes due to less contamination (see also Vujičić *et al.* 2011). They found that some sterilizing treatments could cause the bryophytes to develop resistance to the toxic effects of the biocide. Vujičić *et al.* (2011) also suggested the use of sugar-free medium for ***Hypnum cupressiforme*** (Figure 9). They found that lower temperatures (18-20°C) also helped.

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

Summary

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

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

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

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

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

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

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

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