Current Clinical Microbiology Reports (2023) 10:214–221
https://doi.org/10.1007/s40588-023-00209-2

REVIEW

Status of Drug Discovery in Wetlands Through a Lens of Bioprospecting for New Antimicrobials Being Produced by Microorganisms

Bojana Stekovic[1] · **Karl Anderson**[1] · **Delainey Lancaster**[1] · **Evelyn Stuck**[1] · **Tsebaot Getachew**[1] · **Venugopal Mukku**[1] · **Brian J. Dingmann**[1]

Accepted: 23 October 2023

Abstract

Purpose of Review The current review focuses on opportunities for new areas and new methods to isolate microorganisms from soil that could lead to new antimicrobial compounds.

Recent Findings Ongoing investigation of wetland microbial communities is vital for identifying novel antibiotics to combat the escalating threat of AMR. Exploring uncultured bacteria in soil has also led to promising compounds like teixobactin, which is effective against drug-resistant bacteria.

Summary Bioprospecting for new antimicrobials in wetland environments that focus on previously unculturable bacteria is an emerging field of study. The need for new antimicrobials demands innovative approaches to discovery, such as the Tiny Earth project, and a collaborative focus, such as the One Health Initiative.

Keywords Antibiotic resistance · Wetlands · Bioprospecting · Unculturable bacteria · Tiny Earth project · One Health

Introduction

Antimicrobial compounds, better known as antibiotics, are frequently used to treat bacterial infections by inhibiting further growth or damaging and killing the bacteria. Today, antibiotic misuse and overuse are widespread, increasing antibiotic resistance in the environment [1–5]. Consequently, antimicrobial resistance (AMR), a significant threat to human health, is increasing worldwide, pressuring the discovery and development of new antibiotics [2, 6, 7]. The growing gap between how fast microbes are developing resistance and the development of new antimicrobials is a public health threat [5] that keeps growing due to the inability to prevent inappropriate antibiotic use, lack of adequate guidelines [8], as well as poor sanitation, and hygiene [9]. "Superbugs," opportunistic strains of bacteria, viruses, parasites, and fungi that are resistant to most of the antibiotics and other medications commonly used to treat infections are rapidly acquiring resistant genes against most of the familiar antimicrobial substances, both of natural and synthetic origin [10]. The best example represents methicillin-resistant *Staphylococcus aureus*, usually referred to as MRSA that became resistant to an entire class of antibiotics called beta-lactams [11]. The World Health Organization (WHO) identified a new "Priority 1: Critical" bacterial group as Gram-negative ESKAPE pathogens, i.e., *Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. [12, 13•]. These bacteria pose a significant threat worldwide as they are observed to be multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant [14], including resistance to oxazolidinones, lipopeptides, macrolides, fluoroquinolones, tetracyclines, β-lactams, β-lactam–β-lactamase inhibitor combinations, and last-line antibiotics including carbapenems, glycopeptides, and polymyxins [8, 13•].

This article is part of the Topical Collection on *Antimicrobial*

✉ Brian J. Dingmann
dingm021@crk.umn.edu

[1] University of Minnesota Crookston, 2900 University Avenue, Crookston, MN 56716, USA

What is the Current Status of Antibiotic Resistance?

MRSA and ESKAPE pathogens' proficiency in expressing resistance to a broad range of antibiotics is influenced by the selective pressure on pathogenic bacteria to acquire an extensive range of antibiotic resistance genes (ARGs) via horizontal gene transfer [15]. For example, a recent study demonstrated that out of 79 samples, a total of seven were a mcr-1 positive *E. coli* (MCREC) strain, which indicates a multidrug-resistant strain with a gene for the type IV secretion system (T4SS). T4SS aids in the plasmid-mediated horizontal transfer of genes encoding virulence and antibiotic resistance [16]. This characteristic causes multidrug resistance to spread more quickly among various populations of pathogens. This situation is exacerbated in many low- and middle-income countries due to limited resources to combat the issues mentioned above for the societal causes of the spread of antibiotic resistance [5].

The resistance to penicillin, an antibiotic that has been treating bacterial infections for over 70 years, is, unfortunately, the fate of all antibiotics now on the market. As more microorganisms are acquiring resistance to existing antibiotics, further discovery and characterization of bacterial genes involved in synthesizing antimicrobial compounds are crucial for developing new antibiotics. The pharmaceutical industry has employed various strategies over the last two decades. The basic strategy involved screening for new active compounds, generally from bacterial-derived natural products, followed by chemical optimization around a scaffolding [17].

Many pharmaceutically essential secondary metabolite natural products are synthesized by polyketide synthase (PKS) and non-ribosomal polypetide synthetases (NPRS), including two crucial microbial secondary products PK and NPR that exhibit antimicrobial, anticancer, and immunosuppressive bioactivities [18, 19]. Additionally, it has been found that the PKS and NRPS gene sequences are dominant in the Actinobacteria genome and specifically more abundant in the *Streptomyces* species [18, 20]. The third most prominent bacterial family, Actinobacteria, has produced over 20,000 secondary metabolites that have shown a wide array of activities as bioactive compounds [10]. This emphasizes the importance of the Actinobacteria bacterial family, with *Streptomyces* being in the spotlight as the genus known for its extensive ability to produce antibiotics [18, 20].

As antimicrobial resistance has become a worldwide phenomenon, the urgency for discovering new bioactive compounds is spreading, and attention is being directed back towards the soil microbiome. Some of the most well-known antibiotics, such as penicillin and streptomycin, were developed from soil microorganisms [21, 22]. Wetlands provide unique and varied soil habitats for microbial diversity due to their physical and chemical characteristics. The absence of oxygen in this environment creates a niche for anaerobic microorganisms not typically found in other environments.

What are Wetlands and What Bacteria Inhabit Them?

Wetlands create surroundings with low oxygen levels, prompting the growth of methanogenic bacteria and sulfate-reducing bacteria. This largely unexplored environment represents a rich source of diverse bacterial communities that produce a range of unique compounds and could be ripe for bioprospecting in the future.

Studies have found that actinomycetes, the most prolific producer of antibiotics [23], are more abundant in wetland soils compared to other soil types and can harbor unique strains of Actinobacteria in wetlands with high levels of organic matter, nitrogen, and phosphorus [24–27]. This provides a source of energy and nutrients for microbial growth, prompting the diversity and abundance of microbial communities in these environments. Many studies have demonstrated the existence of bacterial communities that have not been cultured in the laboratory [28, 29••]. Thus, wetland soils are potentially a significant source of new antibiotics if these previously uncultured bacteria could be cultured in the laboratory.

Microbial communities are integral components of the biota in wetlands and play essential roles in shaping the composition and function of these ecosystems. The variety of wetland microbial communities is tightly linked with plant biota through nutrient cycling, plant-microbe interactions, and direct impacts on plant growth and health [30]. It has been demonstrated that the type of plants present, including vascular plants and bryophytes, significantly influenced microbial diversity, richness, and bacteria community structure [31, 32]. *Sphagnum* peat mosses are a crucial player as they construct *Sphagnum* peat bogs that are one of the prevailing ecosystems in the Northern Hemisphere's boreal and tundra zones and store 30% of total soil organic carbon [33, 34]. This cosmopolitan genus of mosses aids in the formation of peat by amassing organic material, which produces a physical barrier that retains water and nutrients. Combined with high moisture, decreased temperature, low pH, nutrient richness, and anoxic conditions [35], these specific environments were a hotspot for specific bacterial communities that fulfill essential ecosystem functions and health roles [31]. Proteobacteria, Actinobacteria, and Acidobacteria are the predominant bacterial taxonomic groups in peat bogs, followed by Cyanobacteria, Bacteroidetes, and Verrucomicrobia [31•, 36, 37]. In addition to the high functional genetic diversity observed in the *Sphagnum*-associated microbes, many bacterial taxa within these communities have yet to be identified [38, 39]. Studying these environments is crucial for identifying the microbial community diversity and structure because many of these microorganisms produce compounds involved in various biological processes. Additionally, it offers a possibility for new antimicrobial activity investigations and bioprospecting as this diversity produces distinctive compounds such as bacteriocins, antibiotics, and antifungal agents that can be used to counter pathogenic species and drug-resistant bacteria.

A recent study has demonstrated a strong correlation between alterations in soil microbial populations across various regions and changes in soil chemistry [40]. The diversity of microbial communities that inhabit various soil types is significantly impacted by soil properties such as soil pH, temperature, texture, moisture, and amount of organic matter [41, 42]. Generally, soil characteristics are intricately linked to the variety of microbial populations; hence, we can better manage and maintain healthy soils that support diverse and productive ecosystems by understanding how different soil properties impact soil microbiomes.

There is a correlation between bacterial diversity and community composition primarily associated with pH [43], with different groups of organisms thriving in soil with different pH ranges. Relative abundance and distribution of Acidobacteria, a predominant bacterial phylum in wetland soils [41, 44], exhibit distinct and significant reactions to pH changes. For example, Acidobacteria subgroups Gp1-3 were found to be adversely affected by pH [2, 41•, 45, 46] and being abundant in acidic bogs. Similarly, the relative abundance of Alphaproteobacteria and Gammaproteobacteria decreased towards higher pH [47]. In contrast, Acidobacteria subgroups Gp4-7 were positively correlated with higher pH values [45, 46], thus thriving in a less acidic environment. Given that Acidobacteria, as a group, are bacteria that are notoriously difficult to cultivate in the laboratory [30], they remain largely unexplored for antimicrobial production.

While bog wetlands are one example of *Sphagnum*-associated wetland communities with decreased pH values, fens represent the other end of the extreme, with basic or alkaline soil. Fens are found where springs or streams bring groundwater to the surface rich in alkaline and other minerals that lead to unique microbial diversity [37, 48–50]. The most prevalent group in all alkaline soils is the class Actinobacteria, where DNA sequences' abundance decreased as pH levels decreased [51, 52]. Moreover, previous research has established a positive correlation between high levels of proteobacterial groups and greater availability of carbon [53]. In addition, Proteobacteria and Actinobacteria abundance increased in nutrient-rich soils. At the same time, Acidobacteria, categorized as oligotrophic bacteria, are commonly found in nutrient-deficient and highly acidic habitats, as mentioned above [54].

What is the Current Status of Bioprospecting in Wetlands?

A potential source of novel antimicrobial compounds is a diverse range of microorganisms that inhabit wetland ecosystems, including actinomycetes. These Gram-positive filamentous bacteria that belong to the species of Actinobacteria (Actinomycetota) have been shown to produce a wide range of bioactive compounds, including antibiotics, antimicrobial peptides, bacteriocins, as well as antifungals, and anticancer agents [55]. The wetland environment is believed to provide a unique niche for actinomycetes, allowing them to compete with other microorganisms for resources and develop unique biosynthetic pathways for bioactive compound production [56]. Therefore, further research on actinomycetes isolated from wetlands could provide a valuable source of natural antimicrobial agents. A study on the actinobacterial communities in Sanggou Bay and the Swan Lake wetlands on the coast of Weihai, China, found that out of 109 actinobacterial strains, 104 (95.4%) expressed inhibition against at least one indicator strain, namely, *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Candida albicans, Vibrio anguillarum,* and *Vibrio parahaemolyticu* [18]. In 2022, another study aimed to discover the presence of antibacterial activity among wetland Actinobacteria by isolating rare halophilic actinomycetes from hypersaline soils of Algerian inland Wetland Ecosystems "Sebkhas-Chotts." Hypersaline soil ecosystems represent a rich reservoir for the isolation of significant rare halophilic actinomycetes, and isolates collected from this wetland have been found to express high antibacterial activity against *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus* [57]. Furthermore, the *Bacillus* sp. sed 2.2 strain, which was isolated from wetlands in São Leopoldo, Southern Brazil, showed inhibitory activity; the antimicrobial substance produced by this strain was partially purified and found to be active against pathogenic Gram-positive bacteria, including some species of *Listeria* [58]. A wetland sediment isolate, *Bacillus* sp. sed 1.4, was also observed to have antimicrobial activity against *Listeria*. However, there was not a noticeable inhibition against Gram-negative bacteria, yeast, and other Gram-positive species [59]. This study conducted a comprehensive screening to evaluate the hydrolytic enzymes, antibacterial, and anticandidal activities of eight carefully selected actinomycetes isolates from the natural wetland ecosystem. These isolates were further categorized into four rare genera, namely, *Actinomadura, Nocardia, Nonomuraea, and Micromonospora*, collectively called rare actinomyces [60]. Notably, rare actinomycetes, particularly non-*Streptomyces* Actinobacteria, are known for their inherent difficulty in cultivation; however, their potential as valuable sources of diverse secondary metabolites with medicinal properties is well-documented [61]. Remarkably, the screened isolates exhibited significant anticandidal and antibacterial activities against Gram-positive and Gram-negative bacteria [60].

Regarding *Streptomyces* specifically, researchers have identified a wetland-derived *Streptomyces* sp. Actif450 as *Streptomyces malaysiensis* with increasing antibacterial activity against pathogenic staphylococci. In addition, this species showed inhibition against fungal pathogens including molds and yeasts, such as *Arthroderma vanbreuseghemii, Aspergillus fumigatus, A. niger, Candida albicans, C. glabarta, C. krusei, C. parapsilosis, Fusarium oxysporum, F. solani, Microsporum canis, Rhodotorula mucilaginous,* and *Scodapulariopsis candida* [25]. In another study, a collection of 125 *Streptomyces* strains was obtained from Fetzara Lake, an Algerian wetland. This research revealed that the isolated strains exhibited antimicrobial activity against two Gram-positive bacteria, namely *Bacillus subtilis* and *Micrococcus luteus*, and against a Gram-negative bacterium, *Escherichia coli.* Among the isolated *Streptomyces* strains, six exhibited broad-spectrum inhibitory activity against all three indicator strains, indicating significant promise for discovering novel antibiotics [62]. *Streptomyces* species are acknowledged for their remarkable capacity to synthesize diverse biological compounds [11], where over 90% of the possible secondary metabolites, including several clinical antibiotics like erythromycin and streptomycin, are derived from this species [12, 63]. Additionally, 16 *Streptomyces* strains of these species isolated from rhizosphere habitat in the Zhalong wetland were active against the Gram-positive bacterial test strains, *S. aureus* and *B. subtilis*, and ten isolates showed activity against *Pseudomonas aeruginosa* [64].

What are the Challenges, Limitations, and Future Avenues of Research?

A few new antimicrobial compounds have been recently discovered in wetland organisms [60] that can inhibit the growth of drug-resistant bacteria. Ongoing demand has been particularly increased for Gram-negative pathogens with a highly restrictive barrier, limiting the effectiveness of most drugs. The last time a class of antibiotics expressed efficacy against Gram-negative bacteria was developed was in the 1960s, and little progress has been marked since [65]. However, researchers at the University of Tübingen in 2019 described a new drug, darobactin, a class of antibiotics that prevents further growth of Gram-positive pathogens, including MRSA, both in vitro and in animal infection models [66]. One of the main challenges is the need for effective screening methods to isolate potential microbially derived bioactive compounds. Actinobacteria are a diverse group of bacteria, but many have demonstrated a recalcitrant reluctance to be cultured in the laboratory [61]. As a result, screening for new antimicrobial compounds from Actinobacteria requires high-throughput screening methods and innovative cultivation techniques.

An initiative to address the need for high-throughput screening could be answered by a crowdsourcing initiative called Tiny Earth. Dr. Jo Handelsman created it at the University of Wisconsin Madison to isolate and identify potentially new sources of antibiotics and "inspire and train students in the sciences" [67]. The Tiny Earth project is composed now of students across the globe who participate in this antibiotic discovery community dedicated to discovering potential new antimicrobials. According to the Tiny Earth website [67], over 14,000 students have participated, with over 19,000 isolates and at least 16 antibiotic structures identified to date. This initiative, as mentioned, is global and could lead to low- and middle-income countries leading the way towards drug discovery that is not based on the pharmaceutical industry strategies, as mentioned earlier. The additional goal of the initiative to "inspire and train students in the sciences" has the potential to promote scientific literacy and advocacy in all parts of the world. The idea of citizen scientists searching for new antimicrobial compounds worldwide is intriguing and worth pursuing.

A secondary alternative approach to the standard pharmaceutical strategy could involve utilizing techniques to promote cultivating previously uncultured bacteria. Many studies have demonstrated that roughly just 1% of soil bacteria have been cultured to date [28, 29••]. This fact lends itself to be further explored using innovative culturing techniques to improve the recovery rate of soil bacterial strains. Such strategies have produced promising pharmaceutical and environmental potential from previously uncultured bacteria [68]. Exploiting previously uncultured bacteria in the soil led to a promising new antibiotic, teixobactin, and has since been synthesized. The newly uncovered compound has been successful in targeting the cell wall of many bacteria, even some that are resistant to existing antibiotics, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Mycobacterium tuberculosis* [60, 69••]. More uncultured bacteria are likely waiting to be explored for beneficial bioactive compounds in the wetland environment. These innovative techniques can be divided into *in situ* and *ex situ* cultivation strategies. Bacteria may not grow in the laboratory initially because of issues involving "growth initiation factors" only present in intact soil conditions. *In situ* cultivation can overcome this, which generally requires initial incubation in the field for several weeks to months. The alternative *ex situ* method involves environmental soil samples being "conditioned" to various membranes exposed to the soil in the laboratory. Studies have demonstrated more significant recovery of bacterial strains using these methods compared to traditional standard direct plating [28, 29••]. These methods are both cost-effective and have produced promising results. These same studies have demonstrated that the isolation of filamentous Actinobacteria, the microorganisms responsible for most of the naturally derived antimicrobials, is enhanced through these innovative cultivation techniques. The potential to culture previously unculturable bacteria in areas that contain Actinobacteria is an intriguing focus of future research.

Conclusion

Antimicrobial resistance (AMR) poses a global health crisis, necessitating the discovery of new antibiotics. Wetlands host diverse microbial communities with unique traits that make them promising sources of novel antimicrobial compounds. Actinomycetes, in the phylum Actinobacteria, are prolific producers of bioactive compounds, including antibiotics. Wetlands provide a niche environment that fosters the development of distinct biosynthetic pathways in actinomycetes, offering opportunities to isolate natural antimicrobial agents. Microbial community diversity in wetlands is influenced by pH, moisture, organic matter, plant communities, and nutrient availability. Recent studies reveal diverse bacterial taxa in wetlands, such as Proteobacteria, Actinobacteria, and Acidobacteria. Bioprospecting efforts have successfully identified actinomycetes with potent antimicrobial activities against pathogenic bacteria and fungi.

Ongoing investigation of wetland microbial communities is vital for identifying novel antibiotics to combat the escalating threat of AMR. Exploring uncultured bacteria in soils has also led to promising compounds like teixobactin, which is effective against drug-resistant bacteria. The cost-effective and innovative cultivation methods described in this review potentially provide a roadmap for discovering new antimicrobial compounds.

A second complementary approach would be to develop strategies to identify and develop new antimicrobials collaboratively. As mentioned above, the Tiny Earth project has produced results regarding collaboration and combining resources in drug discovery. The aim to "train and inspire students in the sciences" is aspirational; however, it is also critically important to solve or at least mitigate the worst of the antibiotic resistance crisis. To this end, several of the authors in this review are undergraduates being exposed to the nature of science. The One Health initiative represents a similar global approach. According to the Centers for Disease Control and Prevention (CDC) website, One Health "recognizes the connection between the health of people, animals and the environment" [70]. This statement summarizes this review's central message: we must focus on the connection between the environment and the world's health crisis.

Author Contributions Bojana Stekovic, Karl Anderson, and Brian Dingmann wrote the main manuscript text. Delainey Lancaster, Evelyn Stuck, Tsebaot Getachew, and Venugopal Mukku assisted on preparation of the manuscript.

Funding Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR).

Data Availability Not applicable

Compliance with Ethical Standards

Ethical Approval All reported studies/experiments with human and animal subjects performed by the authors have compiled with all applicable ethical standards.

Conflict of Interest The authors declare they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Serwecińska L. Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health. Water. 2020; https://doi.org/10.3390/w12123313.
2. WHO: Antimicrobial Resistance. 2021. Available online: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
3. Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: the most critical pathogens. Pathogens. 2021; https://doi.org/10.3390/pathogens10101310.
4. Zhang Z, Zhang Q, Wang T, Xu N, Lu T, Hong W, Penuelas J, Gillings M, Wang M, Gao W, Qian H. Assessment of global health risk of antibiotic resistance genes. Nat commun. 2022; https://doi.org/10.1038/s41467-022-29283-8.
5. Larsson DJ, Flach CF. Antibiotic resistance in the environment. Nat Rev Microbiol. 2022; https://doi.org/10.1038/s41579-021-00649-x.
6. Algammal A, Hetta HF, Mabrok M, Behzadi P. Emerging multidrug-resistant bacterial pathogens "superbugs": a rising public health threat. Front Microbiol. 2023;
7. León-Buitimea A, Garza-Cárdenas CR, Garza-Cervantes JA, Lerma-Escalera JA, Morones-Ramírez JR. The demand for new antibiotics: antimicrobial peptides, nanoparticles, and combinatorial therapies as future strategies in antibacterial agent design. Front microbiol. 2020;

8. Kalpana S, Lin W-Y, Wang Y-C, Fu Y, Lakshmi A, Wang H-Y. Antibiotic resistance diagnosis in ESKAPE pathogens—a review on proteomic perspective. Diagnos. 2023; https://doi.org/10.3390/diagnostics13061014.
9. Ramay BM, Caudell MA, Cordón-Rosales C, Archila LD, Palmer GH, Jarquin C, Moreno P, McCracken JP, Rosenkrantz L, Amram O, Omulo S, Call DR. Antibiotic use and hygiene interact to influence the distribution of antimicrobial-resistant bacteria in low-income communities in Guatemala. Sci rep. 2020; https://doi.org/10.1038/s41598-020-70741-4.
10. Baral B, Mozafari MR. Strategic moves of “superbugs” against available chemical scaffolds: signaling, regulation, and challenges. ACS Pharmacol Translat Sci. 2020; https://doi.org/10.1021/acsptsci.0c00005.
11. Algammal AM, Hetta HF, Elkelish A, Alkhalifah DHH, Hozzein WN, Batiha GES, El Nahhas N, Mabrok MA. Methicillin-resistant Staphylococcus aureus (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infect Drug Resist. 2020; https://doi.org/10.2147/idr.s272733.
12. De Oliveira, D. M., Forde, B. M., Kidd, T. J., Harris, P. N., Schembri, M. A., Beatson, S. A., PAterson, D. L., & Walker, M. J. Antimicrobial resistance in ESKAPE pathogens. Clin microbiol rev, 2020 https://doi.org/10.1128/CMR.00181-19
13.• Denissen J, Reyneke B, Waso-Reyneke M, Havenga B, Barnard T, Khan S, Khan W. Prevalence of ESKAPE pathogens in the environment: antibiotic resistance status, community-acquired infection and risk to human health. Int J Hygiene Environ Health. 2022 10.1016/j.ijheh.2022.114006; **In this study, researchers aim to investigate the prevalence and antibiotic resistance status of ESKAPE pathogens and assess the associated risk to human health**
14. Panda SK, Buroni S, Swain SS, Bonacorsi A, da Fonseca Amorim EA, Kulshrestha M, Nascimento da Silva LC, Tiwari V. Recent advances to combat ESKAPE pathogens with special reference to essential oils. Front Microbiol. 2022; https://doi.org/10.3389/fmicb.2022.1029098.
15. Alcock BP, Raphenya AR, Lau TT, Tsang KK, Bouchard M, Edalatmand A, Huynh W, Nguyen A-L, Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran H-K, Werfalli RE, Nasir JA, Oloni M, Speicher DJ, Florescu A, Singh B, et al. Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic acids res. 2020;2020 https://doi.org/10.1093/nar/gkz935.
16. Lin H, Chen W, Zhou R, Yang J, Wu Y, Zheng J, Dei S, Wu G, Sun Z, Li J, Chen X. Characteristics of the plasmid-mediated colistin-resistance gene mcr-1 in Escherichia coli isolated from a veterinary hospital in Shanghai. Front Microbiol. 2022:1002827.
17.• Saleem M, Hassan A, Li F, Lu Q, Ponomareva LV, Parkin S, Sun C, Thorson JS, Shaaban KA, Sajid I. Bioprospecting of desert actinobacteria with special emphases on griseoviridin, mitomycin C and a new bacterial metabolite producing Streptomyces sp. PU-KB10–4. BMC microbiol. 2023; https://doi.org/10.1186/s12866-023-02770-8. **This research aims to discover and characterize novel bioactive compounds from actinobacteria, which could have implications for drug discovery, antibiotic development and other biotechnological applications**
18.•• Chen L, Wang Z, Du S, Wang G. Antimicrobial activity and functional genes of actinobacteria from coastal wetland. Current Microbiol. 2021; https://doi.org/10.1007/s00284-021-02560-3. **The study identifies culturable actinobacteria isolated from coastal wetlands with broad-spectrum antimicrobial activities and also emphasizes the importance of gene clusters of polyketide synthase (PKS) and non-ribosomal peptide synthase (NPRS)**
19. Chen L, Du S, Qu WY, Guo FR, Wang GY. Biosynthetic potential of culturable bacteria associated with Apostichopus japonicus. J appl microbiol. 2019; https://doi.org/10.1111/jam.14453.
20. Anggelina AC, Pringgenies D, Setyati WA. Presence of biosynthetic gene clusters (NRPS/PKS) in actinomycetes of mangrove sediment in Semarang and Karimunjawa Indonesia. Environ Nat Res J. 2021; https://doi.org/10.32526/ennrj/19/202100050.
21. Hutchings MI, Truman AW, Wilkinson B. Antibiotics: past, present and future. Curr opin microbiol. 2019; https://doi.org/10.1016/j.mib.2019.10.008.
22. Hashmi MZ, Strezov V, Varma A, editors. *Antibiotics and antibiotics resistance genes in soils: monitoring, toxicity, risk assessment and management*, vol. 51. Springer; 2017. https://doi.org/10.1007/978-3-319-66260-2_1.
23. De Simeis D, Serra S. Actinomycetes: a never-ending source of bioactive compounds—an overview on antibiotics production. Antibiotics. 2021;
24. Zamora-Quintero AY, Torres-Beltrán M, Guillén Matus DG, Oroz-Parra I, Millán-Aguiñaga N. Rare actinobacteria isolated from the hypersaline Ojo de Liebre Lagoon as a source of novel bioactive compounds with biotechnological potential. In: *Microbiology*. (Reading England); 2022. https://doi.org/10.1099/mic.0.001144.
25. Benhadj M, Metrouh R, Menasria T, Gacemi-Kirane D, Slim FZ, Ranque S. Broad-spectrum antimicrobial activity of wetland-derived *Streptomyces* sp ActiF450. EXCLI j. 2020; https://doi.org/10.17179/excli2020-1124.
26. Velho-Pereira S, Kamat NM. Antimicrobial screening of actinobacteria using a modified cross-streak method. India j pharmaceut sci. 2011;
27. Yu J, Zhang L, Liu Q, Qi X, Ji Y, Kim BS. Isolation and characterization of actinobacteria from Yalujiang coastal wetland, North China. Asia Pacific J Trop Biomed. 2015; https://doi.org/10.1016/j.apjtb.2015.04.007.
28.•• Almasi F, Kafshnouchi M, Mohammadipanah F, Hamedi J. Fruit wrapping kraft coated paper promotes the isolation of actinobacteria using ex situ and in situ methods. Folia Microbiol. 2021; https://doi.org/10.1007/s12223-021-00907-8. **The article demonstrates the new ex situ and in situ cultivation methods that were introduced for isolation of actinobacteria**
29.•• Jung D, Machida K, Nakao Y, Owen JS, He S, Kindaichi T, Ohashi A, Aoi Y. Cultivation of previously uncultured sponge-associated bacteria using advanced cultivation techniques: a perspective on possible key mechanisms. Front Marine Sci. 2022; https://doi.org/10.3389/fmars.2022.963277. **This study aims to identify actinomycetes isolates from coastal wetlands as a way of making strides toward the development of new antimicrobial products**
30. Kalam S, Basu A, Ahmad I, Sayyed RZ, El-Enshasy HA, Dailin DJ, Suriani NL. Recent understanding of soil acidobacteria and their ecological significance: a critical review. Front Microbiol. 2020; https://doi.org/10.3389/fmicb.2020.580024.
31.• Wicaksono WA, Cernava T, Berg C, Berg G. Bog ecosystems as a playground for plant-microbe coevolution: bryophytes and vascular plants harbor functionally adapted bacteria. Microbiome. 2021; https://doi.org/10.1186/s40168-021-01117-7. **This study shows the significance of bog ecosystems as a place for plant-microbe coevolution which influences healthy functioning and biodiversity in the ecosystem**
32. Guajardo-Leiva S, Alarcón J, Gutzwiller F, Gallardo-Cerda J, Acuña-Rodríguez IS, Molina-Montenegro M, Crandall KA, Pérez-Losada M, Castro-Nallar E. Source and acquisition of rhizosphere microbes in Antarctic vascular plants. Front microbiol. 2022; https://doi.org/10.3389/fmicb.2022.916210.
33. Carrell AA, Lawrence TJ, Cabugao KGM, Carper DL, Pelletier DA, Lee JH, Jawdy SS, Grimwood J, Schmutz J, Hanson PJ,

Shaw AJ, Weston DJ. Habitat-adapted microbial communities mediate *Sphagnum* peat moss resilience to warming. New Phytol. 2022; https://doi.org/10.1111/nph.18072.

34. Kitson E, Bell NGA. The response of microbial communities to peatland drainage and rewetting A review. Front microbiol. 2020; https://doi.org/10.3389/fmicb.2020.582812.
35. Tveit AT, Kiss A, Winkel M, Horn F, Hájek T, Svenning MM, Wagner D, Liebner S. Environmental patterns of brown moss- and Sphagnum-associated microbial communities. Sci rep. 2020; https://doi.org/10.1038/s41598-020-79773-2.
36. Navratilova J, Navratil J, Hajek M. Medium-term changes of vegetation composition on fens of the rural landscape, tested using fixed permanent plots. Folia Geobotan. 2022; https://doi.org/10.1007/s12224-022-09421-2.
37. Carrell AA, Kolton M, Glass JB, Pelletier DA, Warren MJ, Kostka JE, Iversen CM, Hanson PJ, Weston DJ. Experimental warming alters the community composition, diversity, and N_2 fixation activity of peat moss (Sphagnum fallax) microbiomes. Global change biol. 2019; https://doi.org/10.1111/gcb.14715.
38. Alvarenga DO, Rousk K. Unraveling host–microbe interactions and ecosystem functions in moss–bacteria symbioses. J Experiment Botan. 2022; https://doi.org/10.1093/jxb/erac091.
39. Rusin LY. Metagenomics and biodiversity of sphagnum bogs. Mol Biol. 2016; https://doi.org/10.1134/S0026893316050150.
40. Sadeghi S, Petermann BJ, Steffan JJ, Brevik EC, Gedeon C. Predicting microbial responses to changes in soil physical and chemical properties under different land management. Appl Soil Ecol. 2023; https://doi.org/10.1016/j.apsoil.2023.104878.
41.• Ivanova AA, Beletsky AV, Rakitin AL, Kadnikov VV, Philippov DA, Mardanov AV, Ravin NV, Dedysh SN. Closely located but totally distinct: highly contrasting prokaryotic diversity patterns in raised bogs and eutrophic fens. Microorgan. 2020; https://doi.org/10.3390/microorganisms8040484. **The study aims to investigate and compare the microbial diversity patterns in two different types of wetland ecosystems: raised bogs and eutrophic fens**
42. Zheng Q, Hu Y, Zhang S, Noll L, Böckle T, Dietrich M, Herbold CW, Eichorst SA, Woebken D, Richter A, Wanek W. Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. Soil biol biochem. 2019; https://doi.org/10.1016/j.soilbio.2019.107521.
43. Kang E, Li Y, Zhang X, Yan Z, Wu H, Li M, Yan L, Zhang K, Wang J, Kang X. Soil pH and nutrients shape the vertical distribution of microbial communities in an alpine wetland. Sci Total Environ. 2021; https://doi.org/10.1016/j.scitotenv.2021.145780.
44. Jones RT, Robeson MS, Lauber CL, Hamaday M, Knight R, Fierer N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. ISME J. 2009; https://doi.org/10.1038/ismej.2008.127.
45. Kim HS, Lee SH, Jo HY, Finneran KT, Kwon MJ. Diversity and composition of soil Acidobacteria and Proteobacteria communities as a bacterial indicator of past land-use change from forest to farmland. Sci total environ. 2021; https://doi.org/10.1016/j.scitotenv.2021.148944.
46. Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J. 2010; https://doi.org/10.1038/ismej.2010.58.
47. Malard LA, Anwar MZ, Jacobsen CS, Pearce DA. Biogeographical patterns in soil bacterial communities across the Arctic region. FEMS Microbiol Ecol. 2019; https://doi.org/10.1093/femsec/fiz128.
48. Minayeva TY, Bragg O, Sirin AA. Towards ecosystem-based restoration of peatland biodiversity. Mires Peat. 2017; https://doi.org/10.19189/MaP.2013.OMB.150.
49. Emsens, W. J., van Diggelen, R., Aggenbach, C. J., Cajthaml, T., Frouz, J., Klimkowska, A., Kotowski, W., Kozub, L., Liczner, Y., Seeber, E., Silvennoinen, H., Tanneberger, .F., Vicena, J., Wilk, M., & Verbruggen, E. Recovery of fen peatland microbiomes and predicted functional profiles after rewetting. The ISME j. 2020 https://doi.org/10.1038/s41396-020-0639-x
50. Dedysh SN, Ivanova AA, Begmatov SA, Beletsky AV, Rakitin AL, Mardanov AV, Philippov DA, Ravin NV. Microbiology. 2022;91(6):662–70.
51. Muneer MA, Hou W, Li J, Huang X, Kayani UR, Cai Y, Yang W, Wu L, Ji B, Zheng C. Soil pH: a key edaphic factor regulating distribution and functions of bacterial community along vertical soil profiles in red soil of pomelo orchard. BMC microbiol. 2022; https://doi.org/10.1186/s12866-022-02452-x.
52. Wang C, Zhou X, Guo D, Zhao J, Yan L, Feng G, Gao Q, Yu H, Zhao L. Soil pH is the primary factor driving the distribution and function of microorganisms in farmland soils in northeastern China. Ann Microbiol. 2019; https://doi.org/10.1007/s13213-019-01529-9.
53. Mhete M, Eze PN, Rahube TO, Akinyemi FO. Soil properties influence bacterial abundance and diversity under different land-use regimes in semi-arid environments. Sci Afric. 2020; https://doi.org/10.1016/j.sciaf.2019.e00246.
54. Wang Q, Wang C, Yu W, Turak A, Chen D, Huang Y, Ao J, Jiang Y, Huang Z. Effects of nitrogen and phosphorus inputs on soil bacterial abundance, diversity, and community composition in Chinese fir plantations. Front microbiol. 2018; https://doi.org/10.3389/fmicb.2018.01543.
55. Selim MSM, Abdelhamid SA, Mohamed SS. Secondary metabolites and biodiversity of actinomycetes. J Genet Eng biotechnol. 2021; https://doi.org/10.1186/s43141-021-00156-9.
56. Basik AA, Juboi H, Shamsul SSG, Sanglier JJ, Yeo TC. Actinomycetes isolated from wetland and hill paddy during the warm and cool Seasons in Sarawak, East Malaysia. J Microbiol Biotechnol Food Sci. 2021:774–80.
57. Menasria T, Monteoliva-Sánchez M, Benhadj M, Benammar L, Boukoucha M, Aguilera M. Unraveling the enzymatic and antibacterial potential of rare halophilic actinomycetes from Algerian hypersaline wetland ecosystems. J Basic Microbiol. 2022; https://doi.org/10.1590/0001-3765202120201820.
58. Jankoski PR, Correa APF, Brandelli A, MOTTA, A. S. Biological activity of bacteria isolated from wetland sediments collected from a conservation unit in the southern region of Brazil. Anais da Academia Brasileira de Ciências. 2021; https://doi.org/10.1590/0001-3765202120191269.
59. Cavalini L, Jankoski P, Correa APF, Brandelli A, MOTTA, A. S. Characterization of the antimicrobial activity produced by Bacillus sp. isolated from wetland sediment. Anais da Academia Brasileira de Ciências. 2021; https://doi.org/10.1590/0001-3765202120201820.
60. Benhadj M, Gacemi-Kirane D, Menasria T, Guebla K, Ahmane Z. Screening of rare actinomycetes isolated from natural wetland ecosystem (Fetzara Lake, northeastern Algeria) for hydrolytic enzymes and antimicrobial activities. J King Saud University-Sci. 2019; https://doi.org/10.1016/j.jksus.2018.03.008.
61. Ezeobiora CE, Igbokwe NH, Amin DH, Enwuru NV, Okpalanwa CF, Mendie UE. Uncovering the biodiversity and biosynthetic potentials of rare actinomycetes. Future J Pharmaceut Sci. 2022; https://doi.org/10.1186/s43094-022-00410-y.
62. Benhadj M, Gacemi-Kirane D, Toussaint M, Hotel L, Bontemps C, Duval RE, Aigle B, Leblond P. Diversity and antimicrobial activities of Streptomyces isolates from Fetzara Lake, north eastern Algeria. Annales de Biologie Clinique. 2018; https://doi.org/10.1684/abc.2017.1316.
63. Donald L, Pipite A, Subramani R, Owen J, Keyzers RA, Taufa T. Streptomyces: Still the biggest producer of new natural

secondary metabolites, a current perspective. Microbiol Res. 2022; https://doi.org/10.3390/microbiolres13030031.

64. Li Y, Li Y, Li Q, Gao J, Wang J, Luo Y, Fan X, Gu P. Biosynthetic and antimicrobial potential of actinobacteria isolated from bulrush rhizospheres habitat in Zhalong Wetland China. Archiv microbiol. 2018; https://doi.org/10.1007/s00203-018-1474-6.

65. Lehman KM, Grabowicz M. Countering gram-negative antibiotic resistance: recent progress in disrupting the outer membrane with novel therapeutics. Antibiot. 2019; https://doi.org/10.3390/antibiotics8040163.

66. Imai Y, Meyer KJ, Iinishi A, Favre-Godal Q, Green R, Manuse S, Caboni M, Mori M, Niles N, Ghiglieri M, Honrao C, Ma X, Guo JJ, Makriyannis A, Linares-Otoya L, Bohringer N, Wuisan ZG, Kaur H, Wu R, et al. A new antibiotic selectively kills Gram-negative pathogens. Nat. 2019; https://doi.org/10.1038/s41586-019-1791-1.

67. Tiny Earth Network. Studentsourcing Drug discovery. (n.d.). https://tinyearth.wisc.edu.

68. Bodor A, Bounedjoum N, Vincze GE, Erdeiné Kis Á, Laczi K, Bende G, Szilágyi A, Kovács T, Perei K, Rákhely G. Challenges of unculturable bacteria: environmental perspectives. Rev Environ Sci Bio Technol. 2020; https://doi.org/10.1007/s11157-020-09522-4.

69.•• Shukla R, Lavore F, Maity S, Derks MG, Jones CR, Vermeulen BJ, Meclarová A, Morris MA, Becker LM, Wang X, Kumar R, Mederios-Silva J, van Beekveld RAM, Bonvin AMJJ, Lorent JH, Lelli M, NOwick JS, MacGillavry HD, Peoples AJ, et al. Teixobactin kills bacteria by a two-pronged attack on the cell envelope. Nat. 2022; **This study describes the mode of action of an antimicrobial agent identified using new cultivation techniques**

70. Centers for Disease Control and Prevention (n.d.). One Health. One Health | CDC