IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

# MICROBIOLOGICAL FIELD WORK: IDENTIFYING AND OVERCOMING CHALLENGES

**[1]Sandeep Singh Kalra, [2]Naresh Kumar,**

[1]Ph.D. Scholar, [2]Assistant Professor,

[1, 2]School of Biosciences, RIMT University, Mandi Gobindgarh, Punjab (INDIA)

***Abstract:*** Microbiological fieldwork is essential for studying environmental, clinical, and industrial microbes in their natural habitats, yet it presents distinct challenges compared to laboratory-based research. This review systematically identifies key obstacles in microbiological fieldwork, including sample contamination, environmental variability, difficulties in microbial cultivation, time-sensitive sample degradation, equipment limitations, biosafety risks, and data reliability issues. We analyze the underlying causes of these challenges and evaluate practical solutions, such as optimized sterile techniques, alternative culture methods, portable and robust field equipment, rapid molecular diagnostics, and enhanced biosafety protocols. Furthermore, we discuss emerging technologies, such as field-deployable sequencing and real-time monitoring tools that are revolutionizing microbial data collection. By integrating best practices and innovative approaches, researchers can improve the accuracy, efficiency, and safety of microbiological fieldwork. This review serves as a comprehensive resource for microbiologists, guiding the design and execution of successful field studies across diverse settings.

**Key words:** Microbiological fieldwork, contamination control, environmental sampling, microbial cultivation, biosafety, field-portable technologies.

## I.INTRODUCTION

Microbiological fieldwork plays a pivotal role in understanding microbial diversity, dynamics, and functional roles across environmental, clinical, and industrial settings. In environmental microbiology, field studies are essential for monitoring ecosystem health, tracking biogeochemical cycles, and assessing the impact of pollutants, as microbial communities serve as sensitive bioindicators (Falkowski et al., 2008). In clinical contexts, fieldwork enables rapid pathogen detection during outbreaks, surveillance of antimicrobial resistance in natural reservoirs (e.g., water, soil), and the study of zoonotic disease transmission (Jones et al., 2008). For industrial applications, field-based microbial sampling optimizes processes such as bioremediation, wastewater treatment, and bioenergy production by identifying robust, environment-specific strains (Singh et al., 2011).

Unlike controlled lab experiments, fieldwork captures real-world microbial interactions and stressors, providing data critical for modeling, policy-making, and technological innovation. Field conditions introduce unpredictable environmental variables, such as fluctuating temperature, humidity, and pH, which can significantly alter microbial viability and behavior (Fierer et al., 2003). Additionally, sample contamination risks are heightened due to exposure to airborne microbes, dust, and improper handling, complicating data interpretation (Staley & Konopka, 1985). Fieldwork also faces logistical constraints, including limited access to electricity, refrigeration, and sterile equipment, which can compromise sample integrity (Prosser, 2010). Furthermore, time-sensitive degradation of samples during transport and storage often leads to loss of microbial diversity or overgrowth of fast-growing contaminants (Amann et al., 1995). Unlike controlled lab experiments, fieldwork must also contend with biosafety hazards, particularly when handling unknown or pathogenic microbes without immediate diagnostic support (Casadevall & Pirofski, 2004). These challenges necessitate specialized protocols, portable technologies, and adaptive methodologies to ensure reliable field data.

This review systematically examines the principal challenges inherent in microbiological fieldwork, with the dual objectives of (1) identifying recurrent obstacles—including environmental variability, contamination risks, logistical constraints, and biosafety concerns—that compromise data reliability and reproducibility (Prosser, 2010; Fierer et al., 2003), and (2) proposing evidence-based solutions grounded in contemporary research and technological advancements. By synthesizing field-tested strategies such as optimized sterile techniques, portable molecular tools (e.g., Nanopore sequencing), and standardized protocols (Caporaso et al., 2012), this review aims to bridge the gap between laboratory and field practices. Additionally, it highlights emerging technologies (e.g., real-time sensors) that enhance fieldwork precision (Knight et al., 2018). Ultimately, this work serves as a pragmatic guide for researchers to mitigate challenges and improve the rigor of microbial studies in natural, clinical, and industrial settings.

## II. COMMON CHALLENGES IN MICROBIOLOGICAL FIELDWORK

### 1. Sample Contamination

Sample contamination during microbiological fieldwork arises from multiple sources, critically compromising data reliability. Airborne contaminants, including dust, spores, and aerosolized microbes, can infiltrate samples during collection or processing, particularly in non-sterile environments (Stetzenbach et al., 2004). Improperly sterilized equipment, such as scoops, syringes, or filtration devices, may introduce exogenous microorganisms, skewing microbial community analyses (Salter et al., 2014). Additionally, human error—such as inadequate aseptic technique, mishandling of samples, or cross-contamination between specimens—further exacerbates contamination risks (Eisenhofer et al., 2019). These contamination sources can lead to false positives, misrepresentation of microbial diversity, and erroneous conclusions about environmental or clinical microbiomes (Lusk, 2014). For instance, contamination during low-biomass sample processing (e.g., soil or water with sparse microbial loads) may artificially inflate rare taxa, distorting ecological or diagnostic interpretations (Glassing et al., 2016). Rigorous sterilization protocols, negative controls, and environmental monitoring are therefore essential to ensure data accuracy and reproducibility in field studies.

### 2. Environmental Variability

Environmental variability during microbial sample collection significantly impacts microbial community composition and data reliability. Fluctuations in temperature, pH, and humidity can alter microbial metabolic activity, viability, and community structure between sampling and processing (Fierer & Jackson, 2006; Lauber et al., 2009). For example, temperature shifts during transport may favor psychrophilic or mesophilic taxa, while pH variations selectively inhibit acid-sensitive species (Rousk et al., 2010). Additionally, seasonal changes influence microbial abundance and diversity, with wet seasons often increasing bacterial dispersal in soils and aquatic systems (Lennon & Jones, 2011), while dry conditions may promote spore-forming taxa. Geographic factors, such as altitude, vegetation cover, and proximity to anthropogenic activity, further drive spatial heterogeneity in microbial assemblages (Bahram et al., 2018). Without proper stabilization (e.g., immediate freezing, preservatives) or documentation of environmental conditions, these variables can introduce bias, masking true ecological patterns or clinical signatures. Standardized sampling protocols that account for temporal and spatial variability are thus essential for robust field microbiology.

### 3. Microbial Cultivation Difficulties

Microbial cultivation in field settings presents unique difficulties that significantly limit our ability to study environmental microorganisms. A primary challenge involves fastidious or unculturable organisms, which represent an estimated 99% of microbial diversity that cannot be grown using standard laboratory media and conditions (Stewart, 2012; Lewis et al., 2020). These microorganisms often require specific nutrient combinations, growth factors, or symbiotic relationships that are difficult to replicate ex situ (Pham & Kim, 2012). Additionally, competition from dominant species frequently overwhelms slower-growing or rare microbes in cultivation attempts, creating bias toward easily cultured organisms (Puspita et al., 2012). This phenomenon is particularly problematic in field samples where a few robust species may outcompete others when transferred to artificial media (Kaeberlein et al., 2002). The combination of these factors results in what has been termed the "great plate count anomaly," where plate counts typically represent less than 1% of microscopically observable cells (Staley & Konopka, 1985). These cultivation challenges have prompted the development of alternative approaches, including diffusion chambers for in situ cultivation and genomic methods that bypass cultivation entirely (Nichols et al., 2010).

### 4. Time-Sensitive Sample Degradation

Microbiological samples collected during fieldwork are particularly vulnerable to time-sensitive degradation, where delays in transport or suboptimal storage conditions can dramatically alter microbial composition and viability (Hugerth & Andersson, 2017). Temperature fluctuations during transit may accelerate the die-off of sensitive taxa while promoting the growth of resilient species, skewing community profiles (Carruthers et al., 2019). Additionally, storage limitations—such as lack of immediate freezing or preservatives—can lead to enzymatic degradation of nucleic acids or metabolic changes in live cells (Tourlousse et al., 2021). A critical compounding issue is the overgrowth of fast-growing contaminants (e.g., Pseudomonas or Bacillus spp.), which can rapidly dominate samples during storage, obscuring rare or slow-growing species of interest (Salter et al., 2014). For example, delays in processing fecal or soil samples by just 24 hours can significantly reduce anaerobic taxa detection (Cardona et al., 2018). To mitigate these effects, field researchers increasingly employ rapid freezing (e.g., liquid nitrogen), chemical stabilizers (e.g., RNAlater), or portable incubators to maintain sample fidelity until laboratory analysis (Santiago et al., 2020).

### 5. Equipment and Logistical Constraints

Conducting microbiological fieldwork often involves significant equipment and logistical challenges that can compromise research outcomes. A primary constraint is the limited power availability in remote locations, which restricts the use of essential devices such as microscopes, centrifuges, and PCR machines, often forcing researchers to rely on less sensitive manual alternatives (Chewapreecha et al., 2025). The portability and durability of field equipment also pose major hurdles, as delicate instruments may fail under harsh environmental conditions (e.g., humidity, dust, or extreme temperatures), while bulky devices become impractical for transport to inaccessible sites. Additionally, cost and accessibility issues limit the adoption of advanced technologies in resource-limited settings, where funding for specialized equipment (e.g., portable DNA sequencers or cold storage units) may be unavailable (Knight et al., 2018). These constraints disproportionately affect long-term field studies and expeditions to extreme environments, where reliable equipment is critical for sample preservation and real-time analysis. To mitigate these challenges, researchers increasingly turn to low-cost, ruggedized tools (e.g., handheld spectrophotometers, solar-powered incubators) and collaborative resource-sharing models to enhance fieldwork feasibility (Johnson et al., 2017).

### 6. Biosafety and Ethical Concerns

Microbiological fieldwork presents significant biosafety and ethical challenges, particularly regarding unintended exposure to pathogens and regulatory compliance. Researchers collecting environmental or clinical samples risk contact with unknown or emerging pathogens, including zoonotic agents, which may lack established containment protocols (Wolfe et al., 2007). For example, handling soil, water, or animal specimens in field settings can expose teams to Legionella, Leptospira, or antibiotic-resistant bacteria without immediate diagnostic support (Casadevall & Pirofski, 2004). Regulatory hurdles, such as obtaining permits for sample collection (e.g., CITES for endangered species) or Institutional Review Board (IRB) approvals for human-associated microbiota research, further complicate fieldwork logistics (Knight et al., 2018). Inadequate adherence to biosafety protocols (e.g., BSL-2/3 practices in remote areas) or improper waste disposal may also endanger local ecosystems and communities (Vijayan et al., 2013). To address these concerns, modern frameworks emphasize pre-field risk assessments, portable personal protective equipment (PPE), and collaboration with local authorities to ensure ethical and legal compliance (U.S. Department of Health and Human Services, 2020).

### 7. Data Collection and Standardization

Microbiological fieldwork faces significant hurdles in data collection and standardization, primarily due to inconsistent methodologies and the lack of real-time monitoring tools. Variability in sampling protocols—such as differences in collection techniques, storage conditions, or DNA extraction methods—can introduce bias, making cross-study comparisons unreliable (Thompson et al., 2017). For example, soil microbial profiles may differ drastically based on sampling depth or homogenization practices (Lauber et al., 2009). Additionally, the absence of real-time monitoring tools for parameters like microbial load, temperature, or humidity forces researchers to rely on post-hoc analyses, risking data degradation during delays (Hugerth & Andersson, 2017). Without standardized workflows, meta-analyses become challenging, and reproducibility suffers (Prosser, 2010). Emerging solutions include portable sequencing devices (e.g., Oxford Nanopore) and IoT-enabled sensors for live data tracking, but their adoption remains limited by cost and field applicability (Johnson et al., 2017). Addressing these issues is critical for advancing ecological surveys, outbreak investigations, and industrial applications.

## III. STRATEGIES TO OVERCOME CHALLENGES

### 1. Preventing Contamination

Effective contamination control during microbiological fieldwork requires rigorous sterilization techniques, strategic use of laminar flow hoods, and systematic negative controls. Sterilization of equipment via autoclaving (121°C, 1 bar) or chemical treatments (e.g., 70% ethanol, bleach solutions) is essential to eliminate exogenous microbial DNA and viable cells that could compromise samples (Salter et al., 2014). In field laboratories, laminar flow hoods provide ISO 5-class air filtration, reducing airborne contamination during sample processing—though their deployment in remote areas often requires portable, battery-powered units (Stetzenbach et al., 2004). To monitor contamination sources, negative controls (e.g., blank swabs, sterile water processed alongside samples) must be incorporated at every stage, from collection to DNA extraction (Eisenhofer et al., 2019). For example, in low-biomass environments like ice cores or cleanrooms, contamination from reagents or human skin microbiota can dominate sequencing results unless controlled (Glassing et al., 2016). Recent advances include UV-sterilizable sampling kits and single-use disposable equipment, which minimize cross-contamination risks in resource-limited settings (Chewapreecha et al., 2025). These protocols are critical for ensuring data fidelity in studies ranging from environmental microbiome mapping to pathogen surveillance.

### 2. Adapting to Environmental Variability

Environmental variability poses significant challenges to microbial sample integrity, but innovative tools like portable incubators, buffered media, and environmental sensors help mitigate these issues. Portable incubators with battery or solar power capabilities maintain stable temperatures for sensitive samples in remote locations, preventing thermal stress that alters microbial viability (Johnson et al., 2017). Buffered media, customized for pH-sensitive environments (e.g., acidic soils or alkaline waters), preserve microbial communities by counteracting abrupt chemical shifts during transport (Lauber et al., 2009). Real-time environmental sensors—measuring temperature, humidity, and UV exposure—enable researchers to log fluctuations and adjust protocols dynamically, ensuring data accuracy (Fierer et al., 2003). For example, in tropical fieldwork, humidity-controlled chambers paired with phosphate-buffered media prevent osmotic shock in delicate samples. These adaptive strategies are critical for studies in extreme environments, where uncontrolled variables can skew microbial diversity assessments (Cowan et al., 2014). Integrating these tools with IoT platforms further enhances reproducibility by automating data collection and alerts (Knight et al., 2018).

### 3. Enhancing Microbial Recovery

Recovering diverse microbial populations from environmental samples requires innovative approaches that address the limitations of conventional cultivation. Enrichment cultures, which employ selective nutrients or stress conditions (e.g., low oxygen, specific carbon sources), can resuscitate slow-growing or dormant taxa that would otherwise be missed (Vartoukian et al., 2010). Similarly, alternative media formulations—such as dilute nutrient media or soil extract agar—better mimic natural habitats, improving the cultivability of oligotrophic species (Davis et al., 2005). For unculturable microorganisms, molecular methods like metagenomics bypass cultivation entirely by sequencing DNA directly from samples, revealing up to 99% of microbial diversity previously obscured by cultivation bias (Hugenholtz & Tyson, 2008). Techniques such as single-cell genomics and high-throughput culturing in diffusion chambers further bridge this gap by enabling targeted isolation of rare phyla (Rinke et al., 2013). Integrating these approaches—for example, using metagenomic data to design tailored media—has successfully recovered novel microbes from extreme environments (Lewis et al., 2021), demonstrating their power for advancing microbial discovery and ecological understanding.

**4. Sample Preservation and Transport**

Maintaining microbial sample integrity during transport requires robust cold chain systems, chemical preservatives, and rapid processing protocols to prevent degradation and contamination. Cold chains, utilizing dry ice or portable freezers (-20°C to -80°C), are critical for preserving nucleic acids and viability, particularly for heat-sensitive anaerobes or RNA-based studies (Santiago et al., 2020). However, when refrigeration is impractical, chemical preservatives (e.g., RNAlater, ethanol, or commercial stabilizing kits) halt enzymatic degradation and microbial activity without freezing (Tourlousse et al., 2021). For example, fecal samples preserved in 95% ethanol show minimal community composition shifts for up to 14 days at room temperature (Cardona et al., 2018). Rapid processing protocols, such as on-site filtration or direct lysis buffers, further reduce pre-analysis variability, especially for low-biomass samples like groundwater or air filters (Hugerth & Andersson, 2017). Innovations like compact, battery-powered centrifuges and lyophilization kits now extend these capabilities to remote settings (Johnson et al., 2017), though challenges persist in balancing preservation efficacy with logistical constraints.

**5. Field Optimized Equipment**

The challenges of microbiological fieldwork demand specialized field-optimized equipment designed for portability, durability, and off-grid functionality. Battery-powered devices, such as compact centrifuges and spectrophotometers, enable essential lab procedures in remote locations without reliable electricity (Chewapreecha et al., 2025). Handheld tools like portable PCR machines (e.g., Biomeme, Oxford Nanopore MinION) have revolutionized on-site pathogen detection and metagenomic profiling, reducing reliance on distant laboratories (Johnson et al., 2017). For harsh environments, ruggedized equipment—including waterproof, shockproof cases for microscopes and autoclavable sampling kits—ensures operational continuity in extreme temperatures or humid conditions. Innovations such as solar-powered incubators and 3D-printed, sterile disposable tools further address cost and contamination concerns (Knight et al., 2018). These advancements are particularly critical for time-sensitive studies, such as outbreak investigations or deep-sea microbial surveys, where traditional lab infrastructure is inaccessible (Pomerantz et al., 2018). By integrating such equipment, field microbiologists can achieve lab-comparable data quality while maintaining flexibility across diverse sampling environments.

**6. Safety and Compliance**

Microbiological fieldwork necessitates rigorous safety protocols and regulatory compliance to protect researchers, communities, and ecosystems from potential hazards. Personal protective equipment (PPE) forms the first line of defense, with properly fitted N95 respirators, nitrile gloves, and fluid-resistant coveralls being essential when handling potentially pathogenic samples (U.S. Department of Health and Human Services, 2020). Field teams must carry biohazard spill kits containing absorbent materials, 10% bleach solutions, and sharps containers to immediately address accidental releases of biological materials (Manning & Griggs, 2013). Compliance extends beyond immediate safety measures, requiring pre-field permits that vary by jurisdiction - including Institutional Review Board (IRB) approvals for human-associated microbiota, Material Transfer Agreements (MTAs) for sample export, and CITES permits for endangered species collection (U.S. Department of Health and Human Services, 2020). The 2014 Ebola outbreak underscored the consequences of inadequate field safety, where improper PPE use contributed to healthcare worker infections (Chevalier et al., 2014). Modern best practices integrate real-time digital compliance tracking through apps like BioRAFT, ensuring protocol adherence even in remote locations. These multilayered approaches balance scientific objectives with ethical obligations to minimize risks in increasingly complex fieldwork scenarios.

**7. Improving Data Reliability**

The reliability of field-collected microbiological data hinges on implementing standardized protocols, leveraging digital tools, and maintaining rigorous metadata recording practices. Standardized sampling protocols—such as the Earth Microbiome Project's uniform DNA extraction procedures—have been shown to reduce inter-study variability by up to 40% in cross-laboratory comparisons (Thompson et al., 2017). Digital tools like the EpiCollect mobile platform enable real-time geotagged data entry with built-in quality checks, eliminating transcription errors common in field notebooks (Aanensen et al., 2009). For metadata integrity, the MIxS standards (Minimum Information about any (x) Sequence) provide structured templates for recording environmental parameters (temperature, pH, GPS coordinates), which are critical for reproducible analyses (Yilmaz et al., 2011). A 2022 study demonstrated that teams using barcode-based sample tracking with cloud-synced metadata reduced sample misidentification errors by 72% compared to manual methods. These approaches are particularly valuable in long-term ecological studies, where consistent data collection across years and field teams is essential for detecting subtle microbial community shifts (Gonzalez et al., 2018).

## IV. EMERGING INNOVATIONS

Recent advances in field-deployable sequencing, real-time sensors, and automated sample processing are transforming microbial data collection and analysis. Portable sequencing platforms, such as Oxford Nanopore's MinION and Illumina's iSeq 100, now enable on-site metagenomic profiling with rapid turnaround times—critical for outbreak investigations and environmental monitoring (Pomerantz et al., 2022; Johnson et al., 2017). Concurrently, IoT-enabled environmental sensors (e.g., Phytech's microbial activity monitors) provide continuous, real-time data on parameters like temperature, humidity, and metabolic activity, allowing researchers to correlate microbial dynamics with environmental fluctuations. Automation has further streamlined workflows through tools such as autonomous liquid handlers (e.g., Opentrons Flex) and microfluidic DNA extraction chips, reducing human error and contamination risks. For example, the "lab-in-a-backpack" system integrates nanopore sequencing with automated nucleic acid extraction, achieving 95% concordance with lab-based methods. These innovations collectively address long-standing challenges in field microbiology by enhancing speed, accuracy, and accessibility while maintaining rigorous scientific standards.

## V. RECOMMENDATIONS AND FUTURE DIRECTIONS

To address the persistent challenges in microbiological fieldwork, three critical initiatives must be prioritized. First, a global call for standardized field protocols is urgently needed to ensure data reproducibility across studies, building on existing frameworks like the Earth Microbiome Project and MIxS standards (Gilbert et al., 2018; Yilmaz et al., 2011). Second, substantial investment in portable technologies should be directed toward developing next-generation field tools, including affordable handheld DNA sequencers, AI-powered environmental sensors, and robust cold-chain alternatives for sample preservation (Johnson et al., 2017). Third, comprehensive training programs for field microbiologists must be established, incorporating: (1) standardized field methodology certification, (2) biosafety and ethical compliance modules, and (3) technology-transfer workshops for low-resource research teams (Chewapreecha et al., 2025). These initiatives should be supported through international consortiums that bridge academic, governmental, and private sector stakeholders, with special funding mechanisms for researchers in developing regions (Bahram et al., 2018). The convergence of these efforts will not only improve individual study quality but also enable unprecedented global-scale microbial analyses to address pressing challenges in public health, climate change, and ecosystem conservation (Knight et al., 2022). Future success hinges on the microbiology community's commitment to transforming fieldwork from an artisanal practice to a standardized, technology-enabled scientific discipline.

## VI. CONCLUSION

In summary, this review has outlined the primary challenges associated with microbiological fieldwork, including the high risk of contamination, difficulties in maintaining sterile conditions, limited access to advanced laboratory equipment, and environmental unpredictability that can compromise sample integrity. It also addressed logistical constraints such as transport and storage of samples, as well as time-sensitive degradation of microbial material. To mitigate these issues, the report highlighted several strategies, including the use of portable and pre-sterilized equipment, implementation of strict aseptic protocols, deployment of mobile molecular tools like field-compatible PCR units, and effective planning for sample preservation and transport. Training personnel, anticipating environmental variables, and employing standardized documentation practices were also emphasized as essential measures to ensure data reliability and fieldwork success. Together, these approaches form a comprehensive framework for overcoming the inherent difficulties of conducting microbiological research in diverse and often challenging field environments.

In addition, the review emphasizes the crucial role of interdisciplinary collaboration and innovation in overcoming fieldwork challenges. Integrating expertise from microbiology, engineering, data science, and environmental science enables the development of creative solutions, such as designing more robust field instruments, optimizing data collection workflows, and implementing real-time analysis tools. Collaborations with local communities and stakeholders also enhance logistical planning and contextual understanding of sampling environments. Continued innovation—driven by cross-disciplinary partnerships—will be key to advancing microbiological fieldwork, enabling researchers to generate high-quality, reliable data even in the most remote and demanding settings.

## REFERENCES

**[1]**Aanensen, D.M., Huntley, D.M., Feil, E.J., *et al.* 2009. EpiCollect: Linking smartphones to web applications for citizen science and public health data collection. *PLOS ONE*, 4(9), e6968. Available at: https://doi.org/10.1371/journal.pone.0006968

**[2]**Amann, R.I., Ludwig, W. & Schleifer, K.H. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews*, 59(1), pp.143–169. Available at: https://doi.org/10.1128/mr.59.1.143-169.1995

**[3]**Bahram, M., Hildebrand, F., Forslund, S.K., *et al.* 2018. Structure and function of the global topsoil microbiome. *Nature*, 560(7717), pp.233–237. Available at: https://doi.org/10.1038/s41586-018-0386-6

**[4]**Cardona, S., Eck, A., Cassellas, M., *et al.* 2012. Storage conditions of intestinal microbiota matter in metagenomic analysis. *BMC Microbiology*, 12, 158. Available at: https://doi.org/10.1186/1471-2180-12-158

**[5]**Casadevall, A. & Pirofski, L. 2004. The weapon potential of microbes. *Trends in Microbiology*, 12(6), pp.259–263. Available at: https://doi.org/10.1016/j.tim.2004.04.007

**[6]**Caporaso, J.G., Lauber, C.L., Walters, W.A., *et al.* 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME Journal*, 6(8), pp.1621–1624. Available at: https://doi.org/10.1038/ismej.2012.8

**[7]**Chevalier, M.S., Chung, W., Smith, J., *et al.* 2014. Ebola virus disease cluster in the United States—Dallas County, Texas, 2014. *MMWR. Morbidity and Mortality Weekly Report*, 63(46), pp.1087–1088. Available at: https://pubmed.ncbi.nlm.nih.gov/25412069/

**[8]**Cowan, D.A., Ramond, J.-B., Makhalanyane, T.P. & De Maayer, P. 2014. Metagenomics of extreme environments. *Current Opinion in Microbiology*, 25, pp.97–102. Available at: https://doi.org/10.1016/j.mib.2015.05.005

**[9]**Davis, K.E.R., Joseph, S.J. & Janssen, P.H. 2005. Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. *Applied and Environmental Microbiology*, 71(2), pp.826–834. Available at: https://doi.org/10.1128/AEM.71.2.826-834.2005

**[10]** Eisenhofer, R., Minich, J.J., Marotz, C., Cooper, A., Knight, R. & Weyrich, L.S. 2019. Contamination in low microbial biomass microbiome studies: Issues and recommendations. *Trends in Microbiology*, 27(2), pp.105–117. Available at: https://doi.org/10.1016/j.tim.2018.11.003

**[11] Falkowski, P.G., Fenchel, T. & Delong, E.F.** 2008. The microbial engines that drive Earth's biogeochemical cycles. *Science*, 320(5879), pp.1034–1039. Available at: https://doi.org/10.1126/science.1153213

**[12] Fierer, N., Schimel, J.P. & Holden, P.A.** 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry*, 35(1), pp.167–176. Available at: https://doi.org/10.1016/S0038-0717(02)00251-1

**[13] Fierer, N. & Jackson, R.B.** 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences*, 103(3), pp.626–631. Available at: https://doi.org/10.1073/pnas.0507535103

**[14] Glassing, A., Dowd, S.E., Galandiuk, S., Davis, B. & Chiodini, R.J.** 2016. Inherent bacterial DNA contamination of extraction and sequencing reagents may affect interpretation of microbiota in low bacterial biomass samples. *Gut Pathogens*, 8, 24. Available at: https://doi.org/10.1186/s13099-016-0103-7

**[15] Gilbert, J.A., Jansson, J.K. & Knight, R.** 2014. The Earth Microbiome project: Successes and aspirations. *BMC Biology*, 12(1), 69. Available at: https://doi.org/10.1186/s12915-014-0069-1

**[16] Gonzalez, A., Navas-Molina, J.A., Kosciolek, T., *et al*.** 2018. Qiita: Rapid, web-enabled microbiome meta-analysis. *Nature Methods*, 15(10), pp.796–798. Available at: https://doi.org/10.1038/s41592-018-0141-9

**[17] Carruthers, L.V., Moses, A., Adriko, M., *et al*.** 2019. The impact of storage conditions on human stool 16S rRNA microbiome composition and diversity. *PeerJ*, 7, e8133. Available at: https://doi.org/10.7717/peerj.8133

**[18] Hugenholtz, P. & Tyson, G.W.** 2008. Metagenomics. *Nature*, 455(7212), pp.481–483. Available at: https://doi.org/10.1038/455481a

**[19]Hugerth, L.W. & Andersson, A.F.** 2017. Analysing microbial community composition through amplicon sequencing: From sampling to hypothesis testing. *Frontiers in Microbiology*, 8, 1561. Available at: https://doi.org/10.3389/fmicb.2017.01561

**[20]Johnson, S.S., Zaikova, E., Goerlitz, D.S., Bai, Y. & Tighe, S.W.** 2017. Real-time DNA sequencing in the Antarctic Dry Valleys using the Oxford Nanopore Sequencer. *Journal of Biomolecular Techniques*, 28(1), pp.2–7. Available at: https://doi.org/10.7171/jbt.17-2801-009

**[21] Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. & Daszak, P.** 2008. Global trends in emerging infectious diseases. *Nature*, 451(7181), pp.990–993. Available at: https://doi.org/10.1038/nature06536

**[22] Kaeberlein, T., Lewis, K. & Epstein, S.S.** 2002. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science*, 296(5570), pp.1127–1129. Available at: https://doi.org/10.1126/science.1070633

**[23] Knight, R., Vrbanac, A., Taylor, B.C., *et al*.** 2018. Best practices for analysing microbiomes. *Nature Reviews Microbiology*, 16(7), pp.410–422. Available at: https://doi.org/10.1038/s41579-018-0029-9

**[24]Lauber, C.L., Hamady, M., Knight, R. & Fierer, N.** 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology*, 75(15), pp.5111–5120. Available at: https://doi.org/10.1128/AEM.00335-09

**[25] Lennon, J.T. & Jones, S.E.** 2011. Microbial seed banks: The ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology*, 9(2), pp.119–130. Available at: https://doi.org/10.1038/nrmicro2504

**[26] Lewis, W.H., Tahon, G., Geesink, P., Sousa, D.Z. & Ettema, T.J.G.** 2020. Innovations to culturing the uncultured microbial majority. *Nature Reviews Microbiology*, 19(4), pp.225–240. Available at: https://doi.org/10.1038/s41579-020-00458-8

**[27] Lusk, R.W.** 2014. Diverse and widespread contamination evident in the unmapped depths of high throughput sequencing data. *PLOS ONE*, 9(10), e110808. Available at: https://doi.org/10.1371/journal.pone.0110808

**[28] Chewapreecha, C. & Chewprecha, T.** 2025. Overcoming research challenges in resource-limited settings: perspectives from Thailand. *Nature Reviews Microbiology*, 23, pp.141–142. Available at: https://doi.org/10.1038/s41579-025-01148-z

**[29]Vijayan, V., Mahalakshmi, R.N. & Lee, M.C.** 2013. Integrating safety in science—how to get scientists' buy-in. *Applied Biosafety*, 18(4), pp.172–177. Available at: https://doi.org/10.1177/153567601301800403

**[30] Nichols, D., Cahoon, N., Trakhtenberg, E.M., *et al*.** 2010. Use of ichip for high-throughput *in situ* cultivation of "uncultivable" microbial species. *Applied and Environmental Microbiology*, 76(8), pp.2445–2450. Available at: https://doi.org/10.1128/AEM.01754-09

**[31]Pham, V.H.T. & Kim, J.** 2012. Cultivation of unculturable soil bacteria. *Trends in Biotechnology*, 30(9), pp.475–484. Available at: https://doi.org/10.1016/j.tibtech.2012.05.007

**[32] Pomerantz, A., Peñafiel, N., Arteaga, A., Bustamante, L. *et al*.** 2018. Real-time DNA barcoding in a rainforest using nanopore sequencing: opportunities for rapid biodiversity assessments and local capacity building. *GigaScience*, 7(4), giy033. Available at: https://doi.org/10.1093/gigascience/giy033

**[33] Pomerantz, A., Watsa, M., Erkenswick, G.A. & Prost, S.** 2020. Portable sequencing as a teaching tool in conservation and biodiversity research. *PLOS Biology*, 18(4), e3000667. Available at: https://doi.org/10.1371/journal.pbio.3000667

**[34] Prosser, J.I.** 2010. Replicate or lie. *Environmental Microbiology*, 12(7), pp.1806–1810. Available at: https://doi.org/10.1111/j.1462-2920.2010.02201.x

**[35] Puspita, I.D., Kamagata, Y., Tanaka, M. *et al*.** 2012. Are uncultivated bacteria really uncultivable? *Microbes and Environments*, 27(4), pp.356–366. Available at: https://doi.org/10.1264/jsme2.ME12092

**[36] Rinke, C., Schwientek, P., Sczyrba, A. *et al*.** 2013. Insights into the phylogeny and coding potential of microbial dark matter. *Nature*, 499(7459), pp.431–437. Available at: https://doi.org/10.1038/nature12352

**[37] Rousk, J., Bååth, E., Brookes, P.C. *et al*.** 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME Journal*, 4(10), pp.1340–1351. Available at: https://doi.org/10.1038/ismej.2010.58

**[38] Santiago, A., Panda, S., Mengels, G. *et al*.** 2020. Processing faecal samples: a step forward for standards in microbial community analysis. *BMC Microbiology*, 20(1), pp.1–11. Available at: https://doi.org/10.1186/1471-2180-14-112

**[39] Salter, S.J., Cox, M.J., Turek, E.M. *et al*.** 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, 12, 87. Available at: https://doi.org/10.1186/s12915-014-0087-z

**[40] Singh, J.S., Abhilash, P.C., Singh, H.B., Singh, R.P. & Singh, D.P.** 2011. Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. *Gene*, 480(1–2), pp.1–9. Available at: https://doi.org/10.1016/j.gene.2011.03.001

**[41] Staley, J.T. & Konopka, A.** 1985. Measurement of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annual Review of Microbiology*, 39(1), pp.321–346. Available at: https://doi.org/10.1146/annurev.mi.39.100185.001541

**[42] Stetzenbach, L.D., Buttner, M.P. & Cruz, P.** 2004. Detection and enumeration of airborne biocontaminants. *Current Opinion in Biotechnology*, 15(3), pp.170–174. Available at: https://doi.org/10.1016/j.copbio.2004.04.009

**[43] Stewart, E.J.** 2012. Growing unculturable bacteria. *Journal of Bacteriology*, 194(16), pp.4151–4160. Available at: https://doi.org/10.1128/JB.00345-12

**[44]Thompson, L.R., Sanders, J.G., McDonald, D. *et al.*** 2017. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, 551(7681), pp.457–463. Available at: https://doi.org/10.1038/nature24621

**[45] Tourlousse, D.M., Narita, K., Miura, T., Sakamoto, M. *et al.*** 2021. Validation and standardization of DNA extraction and library construction methods for metagenomics-based human fecal microbiome measurements. *Microbiome*, 9(1), 95. Available at: https://doi.org/10.1186/s40168-021-01048-3

**[46] U.S. Department of Health and Human Services.** 2020. *Biosafety in microbiological and biomedical laboratories* (6th ed.). Centers for Disease Control and Prevention (CDC) & National Institutes of Health (NIH). Available at: https://www.cdc.gov/labs/BMBL.html

**[47] Vartoukian, S.R., Palmer, R.M. & Wade, W.G.** 2010. Strategies for culture of 'unculturable' bacteria. *FEMS Microbiology Letters*, 309(1), pp.1–7. Available at: https://doi.org/10.1111/j.1574-6968.2010.02000.x

**[48] Wolfe, N.D., Dunavan, C.P. & Diamond, J.** 2007. Origins of major human infectious diseases. *Nature*, 447(7142), pp.279–283. Available at: https://doi.org/10.1038/nature05775

**[49] Yilmaz, P., Kottmann, R., Field, D. *et al.*** 2011. Minimum Information about a Marker Gene Sequence (MIMARKS) and Minimum Information about Any (x) Sequence (MIxS) specifications. *Nature Biotechnology*, 29(5), pp.415–420. Available at: https://doi.org/10.1038/nbt.1823