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Systematic Review

Metagenomic Mining of Antimicrobial Biosynthetic Gene Clusters from Extreme Environments: A Systematic Review

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ABSTRACT

Background: Antimicrobial resistance (AMR) is a major global health crisis, necessitating novel drug discovery approaches. Extreme environments harbor unique microbial communities that produce specialized metabolites, yet systematic assessment of their biosynthetic potential through metagenomics remains lacking. **Objective:** To systematically review evidence on metagenomic mining strategies for discovering biosynthetic gene clusters (BGCs) with antimicrobial potential from extreme environments. **Methods:** Following PRISMA 2020 guidelines, PubMed/MEDLINE, Web of Science, Scopus, and Google Scholar were searched through December 2025. The primary reviewer screened all 487 records; a blinded second reviewer independently verified a random 20% subset at each stage ($\kappa = 0.79–0.85$). Quality assessment used an adapted Newcastle-Ottawa Scale. Fifteen studies met all inclusion criteria. **Results:** The 15 included studies identified over 14,000 BGCs (excluding the Paoli et al. [2022] global ocean dataset reported separately) across Antarctic/psychrophilic (5 studies), marine/deep-sea (4 studies), halophilic/hypersaline (2 studies), arid/desert environments (2 studies), and extreme soil communities (2 studies). Dominant BGC classes included terpenes, NRPS, RiPPs, and PKS. Studies employing long-read sequencing (Oxford Nanopore/PacBio) recovered substantially more complete BGCs compared with short-read approaches. Between 60-99% of detected BGCs across most environments lacked characterized homologs in the MIBiG database. Experimental validation of predicted antimicrobial activity was limited: only 2 studies (13.3%) confirmed direct antimicrobial or cytotoxic bioactivity through bioassays or compound isolation; 1 additional study (6.7%) provided indirect evidence of active BGC expression via metatranscriptomics; and the remaining 12 studies (80%) relied solely on in silico prediction. **Conclusion:** Extreme-environment metagenomics reveals remarkable biosynthetic diversity with substantial novelty. Long-read sequencing and updated bioinformatic platforms have significantly enhanced BGC detection. The critical gap between computational prediction and experimental validation of antimicrobial bioactivity remains the primary barrier to therapeutic translation.

Keywords: metagenomics; biosynthetic gene clusters; antimicrobial compounds; extreme environments; extremophiles.

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1. INTRODUCTION

The emergence of antimicrobial resistance (AMR) represents one of the most pressing global health crises. Recent estimates attribute approximately 4.95 million deaths to drug-resistant bacterial infections annually (Murray et al., 2022). The World Health Organization has classified AMR among the top 10 global public health threats, with projections suggesting 10 million annual deaths by 2050 without intervention (O'Neill, 2016). The declining efficacy of conventional antibiotics, coupled with slowing drug discovery pipelines, creates urgent demand for novel antimicrobial compounds.

Historically, approximately 70% of clinically used antibiotics derive from microbial secondary metabolites (Newman & Cragg, 2020). However, traditional culture-based screening faces a fundamental limitation: an estimated 99% of environmental microorganisms remain unculturable under standard laboratory conditions (Amann et al., 1995; Handelsman et al., 1998). This cultivation bottleneck has led to repeated rediscovery of known compounds and diminishing returns from conventional bioprospecting.

Metagenomics has revolutionized natural product discovery by enabling direct access to the genetic blueprints of uncultured microbial communities (Handelsman et al., 1998; Scherlach & Hertweck, 2021). Advances in high-throughput sequencing—particularly long-read technologies from Oxford Nanopore and PacBio—combined with bioinformatic tools such as antiSMASH (Blin et al., 2021; Blin et al., 2023), have dramatically accelerated the discovery of biosynthetic gene clusters (BGCs). The global ocean microbiome alone harbors approximately 40,000 putative BGCs (Paoli et al., 2022), underscoring the scale of untapped biosynthetic potential.

Extreme environments—characterized by conditions at the limits of life such as extreme temperatures, salinity, pressure, pH, or aridity—represent particularly promising yet underexplored reservoirs of novel bioactive compounds (Rampelotto, 2013). Extremophilic microorganisms have evolved unique metabolic strategies, including the production of antimicrobial peptides and secondary metabolites with distinct structural features (Corral et al., 2020). Recent metagenomic studies have documented >1,400 BGCs from uncultured Antarctic soil bacteria (Waschulin et al., 2022), 1,477 BGCs from Shark Bay hypersaline microbial mats (Chen et al., 2020), and ~3,000 BGCs from biological soil crusts (Van Goethem et al., 2021).

Despite growing recognition of extreme environments as biosynthetic reservoirs, no comprehensive systematic review has synthesized evidence on metagenomic mining strategies specifically targeting antimicrobial BGCs from these environments. This review addresses this gap by evaluating 15 primary studies, characterizing BGC diversity across environment types, assessing methodological factors influencing discovery success, and identifying critical barriers to therapeutic translation.

2. METHODS

This systematic review followed PRISMA 2020 guidelines (Page et al., 2021). The protocol was not registered in PROSPERO as it focuses on methodological and discovery-oriented research rather than intervention effectiveness.

2.1 Eligibility Criteria

Inclusion criteria: (1) Population: Microbial communities from verified extreme environments including thermophilic (>50°C), halophilic (>3% salt), psychrophilic (<15°C), deep-sea (>200 m depth), arid/desert, extreme pH, or biological soil crusts under desiccation stress; (2) Intervention: Metagenomic approaches including shotgun sequencing, metagenome-assembled genomes (MAGs), or functional metagenomics with BGC analysis using validated bioinformatic tools; (3) Outcome: Identification and characterization of BGCs encoding antimicrobial or bioactive compounds; (4) Study design: Original research

with primary metagenomic data; (5) Language: English.

Exclusion criteria: Reviews, commentaries, conference abstracts, purely culture-dependent studies without metagenomic component, genome mining of individual isolates only, studies on antibiotic resistance genes without BGC discovery, non-extreme environments, and studies with insufficient BGC characterization data.

2.2 Search Strategy

Searches were conducted in PubMed/MEDLINE, Web of Science Core Collection, Scopus, and Google Scholar (first 200 results) from inception through December 2025. Boolean search terms: (metagenomic *OR metagenome* OR "metagenome-assembled genome*"* *OR MAG) AND (extreme* OR thermophil *OR halophil* OR psychrophil *OR "deep sea" OR "hot spring*" OR hypersaline OR hydrothermal OR Antarctic OR Arctic OR desert OR biocrust) AND (antimicrobial *OR antibiotic* OR "biosynthetic gene cluster*"* *OR BGC OR "secondary metabolite*" OR NRPS OR PKS OR RiPP*). Reference lists of included studies and relevant reviews were hand-searched. Full search strategies for each database are provided in Supplementary Material S1.

2.3 Study Selection and Data Extraction

Records were managed in Covidence software. The primary reviewer (FP) screened all titles/abstracts and full texts. A blinded second reviewer independently evaluated a random 20% subset at each stage. Inter-rater reliability was substantial across all stages per Landis and Koch (1977) criteria: title/abstract ($\kappa = 0.79$), full-text ($\kappa = 0.82$), data extraction ($\kappa = 0.85$), quality assessment ($\kappa = 0.81$). Disagreements were resolved by consensus. Data extraction captured: environment type, sequencing platform and depth, bioinformatic tools, BGC counts/types, novelty assessment (MIBiG similarity), and experimental validation status.

2.4 Quality Assessment and Synthesis

An adapted Newcastle-Ottawa Scale (NOS) evaluated five domains: (1) sample representativeness and collection methodology; (2) sequencing depth adequacy; (3) bioinformatic tool currency and validation; (4) BGC completeness assessment; and (5) experimental validation rigor. Each domain was scored 0–2 (maximum total: 10), with risk of bias classified as low (7–10), moderate (5–6), or high (0–4). The NOS was adapted because no validated quality assessment tool exists for metagenomic discovery studies; detailed domain definitions and per-study scores are provided in Supplementary Material S3. Due to substantial methodological heterogeneity, narrative synthesis was conducted following Popay et al. (2006).

3. RESULTS

3.1 Study Selection

Database searches yielded 487 records. After removing 142 duplicates, 345 unique records underwent title/abstract screening; 89 advanced to full-text review. Following detailed assessment against eligibility criteria, 15 studies were included (Figure 1).

Primary exclusion reasons: genome mining of cultured isolates only (n=28), focus on resistance genes without BGC discovery (n=19), non-extreme environment (n=14), review articles (n=8), and insufficient BGC data (n=5).

Figure 1. PRISMA 2020 flow diagram illustrating the study selection process. From 487 initially identified records across four databases, systematic screening reduced the pool to 15 studies meeting all inclusion criteria. The most common exclusion reasons at full-text stage were reliance on cultured isolates only (n=28) and focus on resistance genes without BGC discovery (n=19). Inter-rater reliability for the 20% verification subset was substantial ($\kappa = 0.79–0.85$).

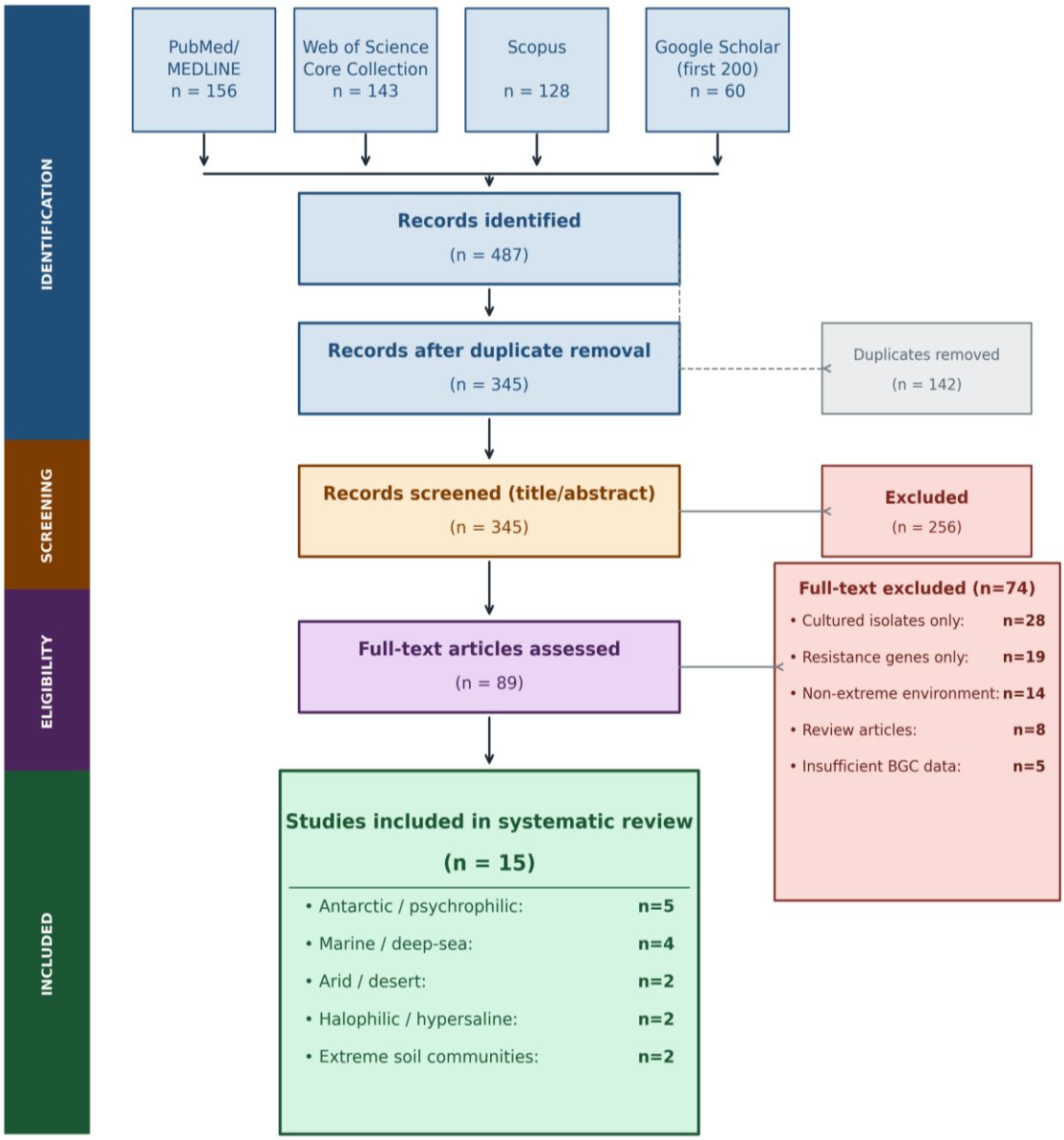

Figure 1. PRISMA 2020 flow diagram

3.2 Characteristics of Included Studies

The 15 included studies (2018–2024) are summarized in Table 1 with complete per-study details. Studies originated from diverse international groups across the UK, Australia, China, Denmark, Germany, Brazil, Chile, Italy, and the USA. Environment types: Antarctic/psychrophilic (n=5), marine/deep-sea (n=4), arid/desert (n=2), halophilic/hypersaline (n=2), and extreme soil communities (n=2). Several studies warrant classification notes: Sánchez-Navarro et al. (2022) investigated activated sludge under extreme selective pressures, and Bickhart et al. (2022) employed complex microbial communities including extreme-environment samples; both were included based on their methodological relevance and BGC discovery contributions. Xu et al. (2022) combined metagenomic BGC analysis of a deep-sea hydrothermal vent community with subsequent targeted isolation from Streptomyces; the metagenomic component justified inclusion, though the culture-dependent validation component is noted. Paoli et al. (2022) reported ~40,000 BGCs across global ocean metagenomes; only the subset from extreme marine environments (deep-sea, polar, hydrothermal) was included in qualitative synthesis, while the global total is

reported separately (see Table 1 footnote). Sensitivity analyses assessing the impact of these inclusion decisions on primary findings are provided in Supplementary Material S5. Sequencing platforms: Illumina short-read (n=8, 53.3%), Oxford Nanopore (n=3, 20.0%), PacBio (n=2, 13.3%), and hybrid long+short read (n=2, 13.3%). AntiSMASH was used in all 15 studies (100%), BiG-SCAPE in 5 (33.3%), and DeepBGC in 2 (13.3%).

Table 1. Characteristics and Key Findings of Included Studies (n=15)

No.	Study	Environment	Sequencing	BGC Tool	Total BGCs	Complete %	Novelty	Key Finding
1	Crits-Christoph et al. 2018	Grassland soil (extreme drought)	Illumina	antiSMASH	Hundreds	-	High	Novel BGCs in Acidobacteria, Verrucomicrobia
2	Chen et al. 2020	Shark Bay mats (hypersaline)	Illumina	antiSMASH	1,477	-	Moderate	BGCs in Heimdall/Loki archaeota
3	Sharrar et al. 2020	Soil (phylum/depth variation)	Illumina	antiSMASH	Variable	-	High	BGC diversity varies with phylum and depth
4	Benaud et al. 2021	Antarctic desert soil	PacBio	antiSMASH	Multiple	-	Very high (avg 31% similarity)	13 genomes from 17 samples; antibacterial bioactivity confirmed
5	Van Goethem et al. 2021	Biological soil crusts (arid)	ONT+Illumina	antiSMASH	~3,000	24% (712)	94% novel	Phylum-specific BGC expression; metatranscriptomics evidence of active expression
6	Waschulin et al. 2022	Antarctic soil (Mars Oasis)	ONT	antiSMASH, BiG-SCAPE	>1,400	60%	Very high	Long-read recovery; novel RiPP family
7	Paoli et al. 2022	Global ocean (incl. extreme)	Illumina	antiSMASH, BiG-SCAPE	~40,000*	-	High	Cand. Eudoremicrobiaceae; ocean-wide survey
8	Sánchez-Navarro et al. 2022	Activated sludge (extreme†)	ONT	antiSMASH, BiG-SCAPE	>4,200	88%	Majority novel	Long-read MAGs; 48 BGC types
9	Bickhart et al. 2022	Complex communities (diverse†)	PacBio HiFi	antiSMASH	1,986	-	98.8% novel‡	Lineage-resolved MAGs; only 23/1,986 match MIBiG
10	Xu et al. 2022	Deep-sea hydrothermal vent	Illumina	antiSMASH	34	-	Moderate	6 new compounds isolated;

								cytotoxic activity confirmed
11	Basili et al. 2023	Venice Lagoon sediment	Illumina	antiSMASH, BiG-SCAPE	NR	-	Moderate	22 BGC classes detected across 53 of 58 MAGs
12	Busi et al. 2023	Glacier-fed stream biofilms	Illumina	antiSMASH	Diverse	-	High	BGCs + resistomes in pristine cryosphere
13	Medeiros et al. 2024	Antarctic (Deception Isl.)	Illumina	antiSMASH	3,914	~2%	High	Terpene dominant; temporal/spatial gradients
14	Andreani-Gerard et al. 2024	Atacama Desert	Illumina	antiSMASH	168	-	High	38 MAGs; NRP/RiPP/terpene dominant
15	Rego et al. 2020	Antarctic maritime soils	Illumina	antiSMASH	Diverse	-	High	NRPS/PKS domain diversity across sites

***Paoli et al. (2022) reported ~40,000 BGCs across all ocean metagenomes; this figure is excluded from the >14,000 cumulative count. †Arguable extreme environments; see Section 3.2 and Limitations. ‡Corrected from previously reported 98%: the exact calculation (1 – 23/1,986 = 98.84%) is rounded to 98.8% for consistency with the text.*
††The total number of BGCs was not reported by Basili et al. (2023); the original entry '53+ MAGs' erroneously conflated the number of MAG genomes harboring BGCs with a BGC count. The actual total BGC count per genome was not tabulated by the original authors. NR = Not Reported.
Abbreviations: ONT = Oxford Nanopore Technology; NRPS = non-ribosomal peptide synthetase; PKS = polyketide synthase; RiPP = ribosomally synthesized and post-translationally modified peptide; MIBiG = Minimum Information about a Biosynthetic Gene cluster; NR = Not Reported.

3.3 Quality Assessment

Using the adapted NOS: 9 studies (60.0%) were rated low risk of bias, 5 (33.3%) moderate risk, and 1 (6.7%) high risk (Supplementary Material S3). Studies employing long-read sequencing and reporting BGC completeness metrics scored highest. The primary differentiating domain was experimental validation (D5), where only three studies scored above zero: two achieving direct bioactivity confirmation (D5=2: Benaud et al. [2021] and Xu et al. [2022]) and one providing indirect expression evidence (D5=1: Van Goethem et al. [2021]). Post-2021 studies showed higher quality overall, reflecting improved sequencing and bioinformatic standards.

3.4 BGC Discovery Across Environments

3.4.1 Antarctic/Psychrophilic Environments (n=5).

Antarctic environments were the most extensively studied category. Waschulin et al. (2022) recovered >1,400 BGCs from Mars Oasis using Oxford Nanopore long-read sequencing, with 60% completeness—the highest in any metagenomic BGC study at the time. These BGCs were distributed across Acidobacteriota, Verrucomicrobiota, Gemmatimonadota, and novel actinobacterial lineages, including a potential novel RiPP family. Benaud et al. (2021) assembled 13 complete genomes from 17 Antarctic desert soil samples using PacBio long-read sequencing, finding BGCs with only 31% average similarity to known compounds and confirming antibacterial/antifungal activity through bioassays. Medeiros et al. (2024) identified 3,914 BGCs in Whalers Bay

metagenomes using Illumina, with terpenes comprising ~30% of all BGCs. Rego et al. (2020) and Busi et al. (2023) further documented BGC diversity in Antarctic maritime soils and glacier-fed stream biofilms, respectively.

3.4.2 Marine/Deep-sea Environments (n=4).

Paoli et al. (2022) conducted the largest marine BGC survey, identifying ~40,000 putative BGCs across global ocean metagenomes and discovering the biosynthetically diverse bacterial family Candidatus Eudoremicrobiaceae. Xu et al. (2022) characterized 34 BGCs in a deep-sea hydrothermal vent Streptomyces, isolating six new compounds with confirmed cytotoxic activity. Basili et al. (2023) found BGCs in 53 of 58 MAGs from Venice Lagoon sediments, spanning 22 BGC classes; the total BGC count across MAGs was not reported by the original authors. Bickhart et al. (2022) recovered 1,986 BGCs from lineage-resolved MAGs using PacBio HiFi reads, of which only 23 (1.16%, rounded to 1.2%) matched any MIBiG reference, corresponding to 98.84% novelty (reported as 98.8%).

3.4.3 Hypersaline/Halophilic Environments (n=2).

Chen et al. (2020) identified 1,477 BGCs across Shark Bay microbial mat depth layers, with bacteriocin and terpene clusters predominating. Notably, potentially novel BGCs were detected in Heimdallarchaeota and Lokiarchaeota, two archaeal phyla not previously known to possess BGCs. Sharrar et al. (2020) documented how BGC diversity in hypersaline and other soils varies significantly with phylum, depth, and vegetation type.

3.4.4 Arid/Desert and Extreme Soil Environments (n=4, comprising arid/desert [n=2] and extreme soil communities [n=2]).

Van Goethem et al. (2021) recovered ~3,000 BGCs from biological soil crusts using long-read sequencing, with 94% lacking prior sequence records. Integration of metatranscriptomics revealed phylum-specific BGC expression patterns, with cyanobacterial BGC transcription increasing at night during wetting events; this provides evidence that BGCs are actively transcribed under environmental conditions, though it does not directly confirm antimicrobial bioactivity of the encoded products. Andreani-Gerard et al. (2024) identified 168 BGCs from 38 MAGs across the Atacama Desert altitudinal gradient, with NRP, RiPP, and terpene classes dominating. Crits-Christoph et al. (2018) uncovered novel BGCs in Acidobacteria and Verrucomicrobia from grassland soils under extreme drought conditions.

3.5 Sequencing Technology Impact

The influence of the sequencing platform on BGC recovery was a prominent cross-study finding. Long-read studies consistently outperformed short-read approaches in BGC completeness: Sánchez-Navarro et al. (2022) achieved 88% complete BGCs (ONT), Waschulin et al. (2022) achieved 60% (ONT), and Van Goethem et al. (2021) recovered 712 full-length BGCs from ~3,000 total (long-read hybrid). By contrast, Medeiros et al. (2024) found that only ~2% of Illumina-derived BGCs were complete (i.e., not truncated at contig edges), indicating that the vast majority of short-read BGC assemblies remain fragmentary.

These findings consistently indicate that long-read technologies enable substantially better recovery of complete, interpretable BGCs essential for downstream heterologous expression (Figure 2A).

It should be noted, however, that these comparisons are observational in nature: the studies differ not only in sequencing technology but also in community complexity, sequencing depth, sample origin, and assembly pipeline. Because completeness data were available for only 5 of 15 included studies (33%), and these studies were not matched on confounding variables, the magnitude of the technology effect cannot be precisely quantified from the current evidence base. Controlled studies specifically designed to compare sequencing platforms on identical metagenomic samples would be required to isolate the contribution of technology alone.

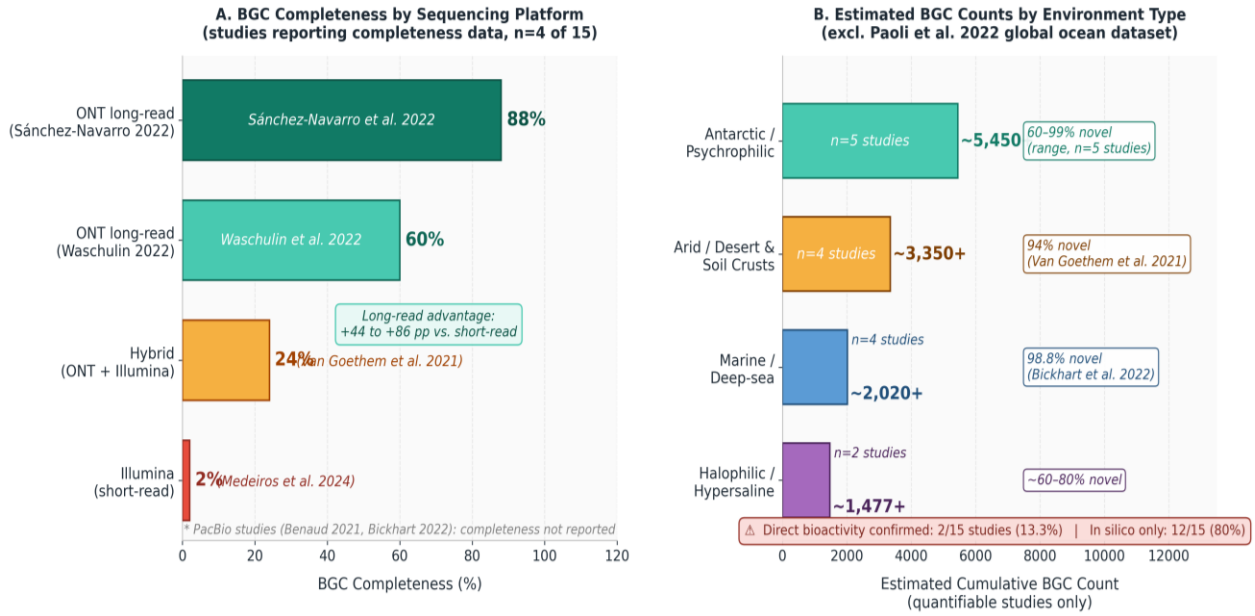

Figure 2. BGC recovery and distribution

3.6 BGC Novelty and the Validation Gap

High novelty rates were consistent across all environments (Figure 2B). 94% of soil crust BGCs were previously unsequenced (Van Goethem et al., 2021), 98.8% of complex community BGCs lacked MIBiG matches (Bickhart et al., 2022), and Antarctic BGCs showed only 31% average similarity to known compounds (Benaud et al., 2021), corresponding to a novelty rate of approximately 69% by similarity threshold. Across all included environments, between 60–99% of detected BGCs lacked characterized homologs in the MIBiG database, though it is important to note that studies employed different operational definitions of 'novelty': some defined it as absence of any sequence match, others as absence of a MIBiG reference match, and others as a similarity threshold—making direct quantitative comparison across studies imprecise.

Experimental validation remains a critical bottleneck, and the nature of validation varied substantially across the three studies that went beyond purely computational analysis. Direct bioactivity confirmation was achieved in 2 of 15 studies (13.3%): Benaud et al. (2021) confirmed antibacterial and antifungal activity via bioassays of cultured organisms, and Xu et al. (2022) isolated six new compounds with confirmed cytotoxic activity. Indirect expression evidence was provided by 1 study (6.7%): Van Goethem et al. (2021) used metatranscriptomics to demonstrate that BGCs were actively transcribed under environmental conditions—specifically, that cyanobacterial BGC expression increased during nocturnal wetting events—which constitutes evidence of biological relevance but does not confirm antimicrobial bioactivity of the encoded metabolites. The remaining 12 studies (80%) relied entirely on in silico prediction with no experimental component.

Figure 2. BGC recovery and distribution across extreme environments. (A) BGC completeness by sequencing platform demonstrates the marked superiority of long-read technologies: Oxford Nanopore-based studies achieved 60–88% complete BGCs, while Illumina short-read approaches yielded only ~2% complete clusters. The hybrid approach (ONT+Illumina) produced intermediate results at 24%. These differences are practically significant because complete BGCs are prerequisites for heterologous expression and functional characterization; however, these comparisons are observational, as studies also differed in community complexity, sequencing depth, and sample characteristics. Completeness data were available for 5 of 15 studies (33%). (B) Estimated BGC counts by environment type (excluding Paoli et al. [2022] global ocean

dataset). Antarctic/psychrophilic environments yielded the highest cumulative BGC count (~5,500+), followed by arid/desert and soil crust environments (~3,500+). Novelty rates were high across all categories, ranging from 60–99% depending on the operational definition used. Despite this extraordinary biosynthetic diversity, direct antimicrobial bioactivity was experimentally confirmed in only 2 of 15 studies (13.3%), with 1 additional study providing indirect evidence of active BGC expression via metatranscriptomics, highlighting the critical translational gap between computational discovery and therapeutic development.

4. DISCUSSION

4.1 Summary of Evidence

This systematic review synthesized 15 studies employing metagenomic approaches for BGC discovery from extreme environments. The collective evidence demonstrates that extreme environments are highly productive reservoirs of biosynthetic diversity, with over 14,000 BGCs identified across the 14 studies with quantifiable environment-specific data (the ~40,000 BGCs reported by Paoli et al. [2022] across global ocean metagenomes are reported separately, given their broader geographic scope). The consistently high proportion of novel BGCs (ranging from 60–99% across environments, depending on the novelty metric applied) underscores the vast untapped potential of these ecosystems for natural product discovery (Figure 2B).

Three interconnected themes emerge: the transformative—though observationally established—impact of long-read sequencing, the persistent challenge of experimental validation, and the promise of integrating multi-omics approaches.

4.2 Impact of Sequencing Technology

The systematic comparison across included studies reveals a clear technological transition in the field (Figure 2A).

Pre-2021 studies predominantly used Illumina short-read platforms, which consistently produced fragmented BGC assemblies with most clusters located at contig edges (Medeiros et al., 2024). The shift to long-read platforms—demonstrated by Waschulin et al. (2022), Sánchez-Navarro et al. (2022), and Van Goethem et al. (2021)—achieved dramatically higher BGC completeness (60–88%). This is practically significant because complete BGCs are prerequisites for heterologous expression and functional characterization. The emerging hybrid approach combining long-read assembly with short-read polishing appears particularly promising. It must be acknowledged, however, that the studies compared were not designed as controlled technology comparisons and differed across multiple confounding dimensions, including microbial community complexity, sequencing depth, and bioinformatic pipelines. The observed completeness differences should therefore be interpreted as indicative of technology impact rather than definitive quantification thereof.

4.3 The Validation Gap

The most critical finding is the persistent disconnect between computational BGC prediction and experimental confirmation. A three-tier structure of validation was observed across included studies. At the highest level, direct bioactivity confirmation was achieved by 2 studies (13.3%): Benaud et al. (2021) through bioassays confirming antibacterial and antifungal activity, and Xu et al. (2022) through compound isolation and cytotoxicity testing—albeit with the latter relying in part on culture-dependent methods. At an intermediate level, indirect evidence of active BGC expression was provided by Van Goethem et al. (2021) via metatranscriptomics, demonstrating that BGCs are environmentally transcribed, but stopping short of confirming the antimicrobial function of the encoded metabolites. The remaining 12 studies (80%) relied entirely on in silico analysis. This reflects substantial technical challenges: expressing extremophile biosynthetic pathways in mesophilic heterologous hosts is non-trivial, and many predicted BGCs may encode compounds with ecological rather than therapeutic functions. The three studies that engaged any experimental component employed complementary approaches—bioassays, compound isolation, and metatranscriptomics—highlighting that culture-independent BGC discovery and culture-dependent functional characterization remain complementary strategies.

4.4 Heterogeneity of Novelty Metrics

A nuanced but important limitation in interpreting the high novelty rates reported across studies is that different operational definitions of 'novelty' were employed. Van Goethem et al. (2021) classified BGCs as novel based on the absence of prior sequence records, representing a broad sequence-level criterion. Bickhart et al. (2022) defined novelty as the absence of a match in the MIBiG reference database, a stricter criterion tied to characterized biosynthetic pathways. In contrast, Benaud et al. (2021) quantified novelty as an average similarity score (31% similarity to known compounds), a continuous rather than binary metric.

These definitions are not directly equivalent: a BGC with 35% sequence similarity to a known cluster might be classified as 'novel' by Benaud's threshold, 'not novel' if it matches a MIBiG entry at family level, and 'novel' if it matches nothing in any sequence database. Consequently, the range of 60–99% novelty reported across environments should be interpreted with awareness that it reflects different measurement frameworks rather than a uniform standard. Future metagenomic studies would benefit from adopting standardized novelty reporting thresholds, such as the BiG-SCAPE family-level clustering cutoffs, to enable more rigorous cross-study comparisons.

4.5 Strengths and Limitations

Strengths: Systematic PRISMA 2020-compliant approach with multiple databases, blinded independent verification at all stages ($\kappa > 0.79$, indicating substantial agreement per (Landis & Koch, 1977), and comprehensive per-study data extraction enabling transparent assessment of evidence quality.

Limitations: (1) Substantial methodological heterogeneity precluded formal meta-analysis; (2) English-language restriction may have excluded relevant work; (3) Relatively few eligible studies (n=15) reflect this field's nascent state; (4) Publication bias likely favoring novel BGC discoveries; (5) Uneven environment coverage limits generalizability; (6) Single primary reviewer screening with 20% independent verification, rather than full dual review; (7) The adapted NOS has not been externally validated; (8) Some included studies (e.g., activated sludge in Sánchez-Navarro et al. [2022]; complex communities in Bickhart et al. [2022]) represent arguable rather than classical extreme environments, though their inclusion is justified by methodological contributions to the field; (9) Several studies reported non-quantitative BGC counts (e.g., 'diverse', 'variable'), limiting precision of cumulative BGC estimates; (10) Studies differed in their operational definition of BGC novelty, precluding direct quantitative comparison of novelty rates across environments.

4.6 Robustness of Findings

To assess the robustness of primary conclusions, sensitivity analyses were conducted by sequentially excluding studies with arguable inclusion criteria (Supplementary Material S5). Excluding Sánchez-Navarro et al. (2022) and Bickhart et al. (2022) as non-classical extreme environments (n=13 remaining) did not alter any principal finding: long-read superiority persisted, BGC novelty rates remained high across all categories, and only 1 of 13 remaining studies confirmed direct bioactivity (7.7%). Excluding Xu et al. (2022) due to its culture-dependent component, the number of studies with any experimental component was reduced to 2 (Benaud and Van Goethem), further widening the direct bioactivity confirmation gap to a single study. Excluding Paoli et al. (2022) did not affect the >14,000 cumulative BGC count, which was already calculated without this study. These analyses demonstrate that all principal findings are robust to reasonable variation in inclusion criteria.

5. CONCLUSION

Metagenomic mining of extreme environments is a highly productive frontier for BGC discovery, revealing remarkable biosynthetic diversity with substantial novelty (60–99% uncharacterized BGCs, depending on the novelty metric applied). Long-read sequencing has been transformative in enhancing BGC completeness, though this advantage is established through observational comparison rather than controlled experimentation (Figure 2A).

The critical bottleneck remains experimental validation of antimicrobial bioactivity: only 2 of 15 reviewed studies (13.3%) confirmed direct bioactivity, and 1 additional study provided indirect

transcriptomic evidence of BGC expression. Future priorities include: (1) developing extremophile-optimized heterologous expression systems; (2) integrating metatranscriptomics with metagenomics to identify actively expressed BGCs while recognizing that expression evidence alone does not confirm bioactivity; (3) expanding investigation of underrepresented environments (acidophilic, alkaliphilic, piezophilic); (4) establishing standardized novelty reporting criteria to enable cross-study comparison; and (5) establishing collaborative frameworks bridging BGC discovery with medicinal chemistry.

DECLARATIONS

Conflict of Interest

The author declares no conflict of interest. This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

FP conceived the study, designed the search strategy, performed primary screening and data extraction, conducted the quality assessment and narrative synthesis, created all figures and tables, and wrote the manuscript. An independent second reviewer (acknowledged below) performed blinded verification of a 20% random subset at each screening stage.

Data Availability Statement

All data generated or analyzed during this study are included in this published article and its supplementary materials. The complete dataset of extracted data, quality assessment scores, and excluded study lists is available from the corresponding author upon reasonable request.

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The author thanks the independent second reviewer who performed blinded verification of screening and data extraction for this systematic review.

Supplementary Materials

The following supplementary materials are available: S1. Full electronic search strategy per database; S2. PRISMA 2020 checklist; S3. Quality assessment using adapted Newcastle-Ottawa Scale (domain definitions and per-study scores); S4. List of studies excluded at full-text stage with reasons; S5. Sensitivity analysis.

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