How have bacterial methylation research methods and understanding of epigenetic mark functions evolved from the 1950s to the present day?

Bacterial methylation research has undergone a clear methodological progression from early targeted biochemical studies to modern genome-wide sequencing approaches, accompanied by an expansion in understanding epigenetic mark functions beyond gene regulation to encompass DNA replication, repair, virulence, and evolutionary adaptation.

Abstract

Bacterial methylation research has advanced from early biochemical and genetic studies to high-resolution genome-wide analyses. Early works (spanning the 1950s to the 1990s) relied on methods such as molecular cloning, pulsed-field gel electrophoresis, and biochemical assays to link methylation with DNA replication, chromosome segregation, repair, and transcription. In later decades, six reviews report the adoption of single-molecule real-time sequencing and five document nanopore sequencing, while seven highlight computational motif analysis and bioinformatics approaches that now enable genome-wide methylome mapping. A minority of studies also describe bisulfite sequencing, machine learning pipelines, and CRISPR-based epigenetic editing.

Reports consistently attribute gene regulation to bacterial methylation (noted in all 10 reviews) and assign additional roles to DNA replication (8 studies), mismatch repair (7 studies), and virulence (7 studies). Recent work expands these functions to include host defense, evolutionary adaptation, epigenetic inheritance, and, in one instance, a novel phosphothioation modification. Overall, the reviewed literature delineates a clear progression in methodology—from targeted, biochemical approaches to integrated, high-throughput techniques—and a parallel broadening in the recognized functional roles of bacterial epigenetic marks.

Paper search

Using your research question "How have bacterial methylation research methods and understanding of epigenetic mark functions evolved from the 1950s to the present day?", we searched across over 126 million academic papers from the Semantic Scholar corpus. We retrieved the 50 papers most relevant to the query.

Screening

We screened in sources that met these criteria:

- **Bacterial Systems**: Does the study focus on bacterial methylation or epigenetic modifications in bacterial systems? This is a mandatory requirement - studies focusing exclusively on eukaryotic (plant, animal, fungal) methylation must be excluded.
- **Methylation Focus**: Does the study substantively discuss DNA methylation, RNA methylation, or other epigenetic modifications? This is a mandatory requirement - studies that only briefly mention methylation without it being a central focus must be excluded.
- **Time Period**: Was the study published between 1950 and August 2025? This is a mandatory requirement as specified by the research question timeframe.
- **Research Type**: Is this a primary research study, systematic review, meta-analysis, or methodological paper? This is a mandatory requirement - editorials, commentaries, news items, and conference abstracts without substantial content must be excluded.

- **Methods/Functions**: Does the study discuss research methods for studying bacterial methylation OR the biological functions of bacterial epigenetic marks? Studies must contribute to understanding of either methodological approaches or functional roles to be relevant.
- **Sufficient Detail**: Does the study provide sufficient methodological or functional detail to assess its contribution to the evolution of the field? Studies with only superficial mentions or insufficient detail may still be included if they represent important historical milestones.
- **Full Text**: Is the full text available or obtainable for detailed analysis? While full text access is strongly preferred for proper assessment of methodological evolution, historically important papers with limited access may still be included if abstracts provide sufficient information.

We considered all screening questions together and made a holistic judgement about whether to screen in each paper.

Data extraction

We asked a large language model to extract each data column below from each paper. We gave the model the extraction instructions shown below for each column.

- **Publication Period**:

  Extract the publication year and identify the decade/era this research represents. If the study references or reviews earlier work, also note the time periods of the foundational research being discussed (e.g., '1970s-1980s work on Dam methylase', '1990s restriction-modification studies', 'early 2000s genomic approaches').

- **Research Methods**:

  Extract all research methods, technologies, and experimental approaches used to study bacterial methylation, including:

- Specific laboratory techniques (biochemical assays, molecular cloning, etc.)
- Sequencing technologies (Sanger, single-molecule real-time, nanopore, etc.)
- Analytical approaches (genome-wide analysis, single-gene studies, comparative genomics)
- Detection methods for methylation (bisulfite sequencing, mass spectrometry, etc.)
- Computational tools and bioinformatics methods
- Note whether methods represent innovations or applications of established techniques

- **Methylation Systems**:

  Identify which specific bacterial methylation systems, enzymes, or mechanisms were the focus of study, including:

- Specific methyltransferases (Dam, Dcm, restriction-modification enzymes)
- Types of methylation (N6-methyladenine, N4-methylcytosine, 5-methylcytosine)
- Target sequences or motifs
- Bacterial species or families studied
- Whether study was system-specific or genome-wide

- **Functional Roles**:

  Extract all functional roles, biological processes, and mechanisms of bacterial methylation that were investigated, discovered, or discussed, including:

- DNA replication and cell cycle control

- Gene regulation and transcriptional control
- Virulence and pathogenesis
- DNA repair and recombination
- Defense against foreign DNA
- Chromosome segregation
- Epigenetic inheritance
- Host-pathogen interactions
- Note whether functions were newly discovered, confirmed, or challenged

- **Key Discoveries**:

  Summarize the main findings, insights, or advances in understanding that this work contributed, including:

- Novel functions or mechanisms discovered
- Connections established between methylation and biological processes
- New understanding of evolutionary or ecological roles
- Methodological breakthroughs or capabilities
- Paradigm shifts or conceptual advances
- How findings built upon or contradicted previous understanding

- **Study Scope**:

  Describe the breadth and approach of the research, including:

- Whether focused on single species/strain or comparative across species
- Lab-based experimental work vs. computational/theoretical analysis
- Mechanism-focused vs. genome-wide vs. systems-level approach
- Basic research vs. applied/clinical focus
- Review/synthesis vs. original empirical research

Results

Characteristics of Included Studies

Study	Study Focus	Time Period Covered	Primary Methods Discussed	Key Functions Identified
Sánchez-Romero et al., 2015	Review of bacterial DNA methylation, historical and current perspectives	1970s–2015 (four decades)	Single-molecule real-time sequencing; genome-wide methylome analysis	Chromosome replication, DNA mismatch correction, transcriptional control, epigenetic lineage formation

Study	Study Focus	Time Period Covered	Primary Methods Discussed	Key Functions Identified
Gao et al., 2023	Review of bacterial DNA methyltransferases, structure, function, and biomedical potential	1970s–2023 (focus on 2000s–2020s)	Single-molecule real-time sequencing, nanopore sequencing, structural biology, synthetic biology, computational motif analysis	Gene regulation, chromosomal replication/repair, virulence, host defense, epigenetic inheritance
Beaulaurier et al., 2018	Review of sequencing technologies for bacterial epigenomes	1970s–2018 (focus on 2010s)	Single-molecule real-time sequencing, nanopore sequencing, genome-wide methylome mapping	Gene regulation, virulence, pathogen-host interactions
Adhikari and Curtis, 2016	Review of Dam and CcrM methyltransferases in gene regulation	Pre-2016 (focus on 2010s)	Literature review; no specific methods detailed	Gene regulation, DNA replication initiation, DNA repair, cell cycle regulation
Chen et al., 2020	Review of bacterial epigenomics in the context of population genomics	Pre-2020 (focus on 2010s–2020s)	Single-molecule real-time sequencing, nanopore sequencing, bisulfite sequencing, motif analysis, bioinformatics	Bacteriophage resistance, replication, stress response, gene regulation, virulence, genome plasticity
Heusipp et al., 2007	Review of DNA adenine methylation in pathogenesis	Pre-2007 (focus on early 2000s)	Literature review; no specific methods detailed	Chromosome replication, mismatch repair, transposition, transcription, virulence gene regulation
Casadesús and Low, 2006	Review of epigenetic gene regulation in bacteria	1970s–2006	Molecular cloning, pulsed-field gel electrophoresis, microarray, computational modeling	DNA replication, chromosome segregation, mismatch repair, gene regulation, virulence, phase variation, epigenetic inheritance

Study	Study Focus	Time Period Covered	Primary Methods Discussed	Key Functions Identified
Casadesús and Torreblanca, 1996	Review of methylation-related epigenetic signals in bacteria	1950s–1990s	Literature review; focus on biochemical and genetic studies	DNA replication, chromosome segregation, mismatch repair, transcriptional regulation
Passeri et al., 2024	Review of epigenomics in bacterial genome evolution	Pre-2024 (focus on 2020s)	Single-molecule real-time sequencing, nanopore sequencing, MeStudio pipeline, machine learning, comparative epigenomics	Defense against foreign DNA, cell cycle, gene expression, virulence, adaptation, epigenetic inheritance
Won and Yim, 2024	Review of methylation-based approaches in microbiome engineering	Early 2000s–2024	Methylation-sensitive restriction enzymes, whole-genome bisulfite sequencing, single-molecule real-time sequencing, nanopore sequencing, CRISPR-based epigenetic editing, bioinformatics	DNA replication, gene regulation, virulence, DNA repair, defense, epigenetic inheritance, microbiome modulation

Across the 10 included reviews of bacterial DNA methylation, we found the following patterns in methods and functions:

- Single-molecule real-time sequencing was discussed in 6 studies.
- Nanopore sequencing was discussed in 5 studies.
- Motif analysis, bioinformatics, or computational approaches were discussed in 7 studies.
- Bisulfite sequencing (including whole-genome bisulfite sequencing) was discussed in 2 studies.
- Literature review without specific methods was found in 3 studies.
- Other molecular or biochemical methods (such as molecular cloning, pulsed-field gel electrophoresis, microarray) were discussed in 4 studies.
- Structural biology, synthetic biology, machine learning, CRISPR-based epigenetic editing, methylation-sensitive restriction enzymes, MeStudio pipeline, and comparative epigenomics were each discussed in 1 study.
- We did not find mention of methods outside these categories.

Key functions identified:

- Gene regulation, transcriptional control, or gene expression was identified as a key function in all 10 studies.
- DNA replication or replication initiation was identified in 8 studies.
- DNA repair, mismatch correction, or mismatch repair was identified in 7 studies.
- Virulence, pathogenesis, or virulence gene regulation was identified in 7 studies.
- Epigenetic inheritance, epigenetic lineage, epigenetic signals, or phase variation was identified in 6 studies.
- Host defense, defense against foreign DNA, or bacteriophage resistance was identified in 4 studies.
- Chromosome segregation and cell cycle/cell cycle regulation were each identified in 2 studies.
- Genome plasticity or adaptation, stress response, transposition, microbiome modulation, and pathogen-host interactions were each identified in 1 study.
- We did not find mention of other key functions outside these categories.

Some studies discussed multiple methods and functions. We did not find any studies that discussed methods or functions outside the categories listed above.

Thematic Analysis

Evolution of Detection and Analysis Methods

Study	Key Methods/Technologies Discussed	Innovations/Breakthroughs
Sánchez-Romero et al., 2015	Single-molecule real-time sequencing; methylome analysis	Enabled genome-wide methylation profiling
Gao et al., 2023	Single-molecule real-time sequencing, nanopore sequencing, structural biology, synthetic biology, computational motif analysis	Genome-wide detection; synthetic epigenetic systems
Beaulaurier et al., 2018	Single-molecule real-time sequencing, nanopore sequencing	Systematic, genome-wide methylation detection
Adhikari and Curtis, 2016	No mention found	No mention found
Chen et al., 2020	Single-molecule real-time sequencing, nanopore sequencing, bisulfite sequencing, motif analysis, bioinformatics	Large-scale, in situ methylation analysis; motif mining
Heusipp et al., 2007	No mention found	No mention found
Casadesús and Low, 2006	Molecular cloning, pulsed-field gel electrophoresis, microarray, computational modeling	Application of microarray and computational models
Casadesús and Torreblanca, 1996	No mention found	No mention found
Passeri et al., 2024	Single-molecule real-time sequencing, nanopore sequencing, MeStudio pipeline, machine learning	Integration of computational pipelines and machine learning

Study	Key Methods/Technologies Discussed	Innovations/Breakthroughs
Won and Yim, 2024	Methylation-sensitive restriction enzymes, whole-genome bisulfite sequencing, single-molecule real-time sequencing, nanopore sequencing, CRISPR-based editing, bioinformatics	CRISPR-based epigenetic editing; high-throughput methylome analysis

Key methods and technologies discussed across the 10 studies included:

- Long-read sequencing (single-molecule real-time sequencing or nanopore sequencing) was discussed in 6 studies.
- Methylome or motif analysis was discussed in 2 studies.
- Bisulfite sequencing, structural biology, synthetic biology, computational motif analysis, molecular cloning, pulsed-field gel electrophoresis, microarray, computational modeling, MeStudio pipeline, machine learning, methylation-sensitive restriction enzymes, whole-genome bisulfite sequencing, and CRISPR-based editing were each discussed in 1 study.
- Bioinformatics approaches were discussed in 2 studies.
- We did not find mention of methods or technologies in 3 studies.

Regarding innovations and breakthroughs:

- Genome-wide methylation detection or profiling was reported as an innovation in 3 studies.
- Synthetic epigenetic systems, large-scale/in situ methylation analysis, motif mining, application of microarray and computational models, integration of computational pipelines and machine learning, CRISPR-based epigenetic editing, and high-throughput methylome analysis were each reported in 1 study.
- We did not find mention of innovations or breakthroughs in 3 studies.

Expanding Understanding of Functional Roles

Study	Key Functional Roles Identified	Novel/Confirmed/Challenged
Sánchez-Romero et al., 2015	Chromosome replication, DNA repair, transcriptional control, epigenetic inheritance	Confirmed, with new insights from methylome analysis
Gao et al., 2023	Gene regulation, replication/repair, virulence, defense, epigenetic inheritance	Confirmed and expanded; biomedical applications highlighted
Beaulaurier et al., 2018	Gene regulation, virulence, host-pathogen interactions	Confirmed, with genome-wide evidence
Adhikari and Curtis, 2016	Gene regulation, DNA replication, repair, cell cycle	Confirmed, focus on Dam/CcrM

Study	Key Functional Roles Identified	Novel/Confirmed/Challenged
Chen et al., 2020	Bacteriophage resistance, metabolism, virulence, genome plasticity, host interaction	Expanded; novel modification (phosphothioation, a novel DNA modification) identified
Heusipp et al., 2007	Replication, repair, transposition, transcription, virulence	Confirmed, focus on virulence regulation
Casadesús and Low, 2006	Replication, segregation, repair, gene regulation, virulence, phase variation, inheritance	Confirmed and expanded; evolutionary convergence
Casadesús and Torreblanca, 1996	Replication, segregation, repair, transcriptional regulation	Confirmed, early synthesis
Passeri et al., 2024	Defense, cell cycle, gene expression, virulence, adaptation, inheritance	Confirmed and expanded; evolutionary/ecological focus
Won and Yim, 2024	Replication, gene regulation, virulence, repair, defense, inheritance, microbiome modulation	Confirmed and expanded; microbiome engineering focus

Across the 10 studies, we found the following functional roles for DNA methylation in bacteria:

- Gene regulation, gene expression, or transcriptional control : 10 studies
- DNA replication or replication initiation : 8 studies
- DNA repair, mismatch correction, or mismatch repair : 7 studies
- Virulence, pathogenesis, or virulence gene regulation : 7 studies
- Epigenetic inheritance, epigenetic lineage, epigenetic signals, or phase variation : 6 studies
- Host defense, defense against foreign DNA, or bacteriophage resistance : 4 studies
- Chromosome segregation and cell cycle/cell cycle regulation : 2 studies each
- Genome plasticity or adaptation, stress response, transposition, microbiome modulation, and pathogen-host interactions : 1 study each

Regarding the type of contribution:

- 9 studies confirmed previously known roles
- 6 studies expanded on previous knowledge
- 1 study identified a novel modification (phosphothioation, a novel DNA modification)
- We did not find any studies that challenged previous roles

Several studies both confirmed and expanded functional roles, and some highlighted new applications or evolutionary/ecological perspectives. We did not find any studies that explicitly challenged established functional roles of DNA methylation in bacteria.

Integration of Epigenomics into Bacterial Biology

Study	Integration with Broader Biological/Evolutionary Context	Systems/Genome-wide/Applied Focus
Sánchez-Romero et al., 2015	Methylome analysis as a new era in epigenomics	Genome-wide
Gao et al., 2023	Methyltransferases as targets for biomedical applications	Genome-wide and applied
Beaulaurier et al., 2018	Large-scale methylome mapping; evolutionary insights	Genome-wide
Adhikari and Curtis, 2016	Mechanistic focus on Dam/CcrM; regulatory diversity	Mechanism-focused
Chen et al., 2020	Population epigenomics; microbiome-host interactions	Genome-wide, ecological
Heusipp et al., 2007	Virulence regulation across pathogens	Mechanism-focused
Casadesús and Low, 2006	Epigenetic inheritance, phase variation, evolutionary convergence	Mechanism and evolutionary
Casadesús and Torreblanca, 1996	Early conceptual expansion of epigenetics to bacteria	Mechanism-focused
Passeri et al., 2024	Epigenomics in genome evolution and adaptation; computational tools	Systems-level, evolutionary
Won and Yim, 2024	Epigenetics as a toolkit for microbiome engineering	Systems-level, applied

Across the 10 studies, we found the following patterns in their integration with broader biological or evolutionary context:

- All 10 studies discussed epigenomics or epigenetics in bacteria.
- 3 studies integrated evolutionary or genome evolution perspectives.
- 4 studies focused on mechanistic or regulatory aspects.
- 2 studies addressed microbiome or host interactions.
- 2 studies included an applied or biomedical context.
- 1 study discussed virulence regulation in pathogens.
- 1 study included computational or tool development aspects.

Regarding systems, genome-wide, and applied focus:

- 4 studies had a genome-wide focus.
- 4 studies had a mechanism-focused approach.
- 2 studies had a systems-level focus.
- 2 studies had an applied focus.
- 2 studies included an explicit evolutionary focus.
- 1 study had an ecological focus.

Some studies were counted in multiple categories if they addressed more than one focus or context.

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