Draft Systematic Review Protocol

# Title

The Human Microbiome and Health: A Systematic Review of Its Role in Gene Regulation and Disease Prevention

# Background

The human microbiome has emerged as a central player in health and disease, influencing immune regulation, metabolic pathways, and even host gene expression. Recent advances in sequencing technologies and systems biology have highlighted microbiome alterations in conditions such as obesity, type 2 diabetes, inflammatory bowel disease, cancer, and neuropsychiatric disorders.  
  
Importantly, microbiota-derived metabolites (e.g., short-chain fatty acids, bile acids, tryptophan derivatives) act as signaling molecules that can regulate host gene expression through epigenetic and transcriptional mechanisms. This suggests a potential for microbiome-targeted interventions (e.g., diet, probiotics, prebiotics, synbiotics, fecal microbiota transplantation, and antibiotics) to prevent or modulate disease pathways.  
  
Despite the growing literature, a comprehensive synthesis of how microbiome modulation affects host gene regulation and disease prevention across humans, animals, and mechanistic models is lacking.

# Objectives

## Primary Objective

To systematically review the evidence on how microbiome composition and modulation influence host gene regulation and disease prevention.

## Secondary Objectives

1. To examine the role of specific microbiota-derived metabolites in regulating host molecular pathways.  
2. To compare effects across different interventions (dietary, probiotic, prebiotic, synbiotic, FMT, antibiotics).  
3. To integrate human, animal, and in vitro evidence into a mechanistic framework of microbiome–host interaction.

# Methods

## Eligibility Criteria

- Study designs: Randomized controlled trials (RCTs), cohort studies, case-control studies, quasi-experimental studies, animal studies, in vitro studies.  
- Population: Humans (all ages, sexes, health conditions), relevant animal models, and in vitro systems.  
- Interventions: Any approach that modulates the microbiome (diet, probiotics, prebiotics, synbiotics, antibiotics, fecal microbiota transplantation, lifestyle interventions).  
- Comparators: Placebo, no intervention, or alternative interventions.  
- Outcomes:  
 - Primary: Evidence of gene regulation (epigenetic changes, transcriptomic shifts, signaling pathway activation).  
 - Secondary: Microbiome composition/diversity changes, disease prevention outcomes, biomarkers of health.  
- Timeframe: No restrictions on publication year.  
- Language: English.

## Information Sources

Electronic databases: PubMed/MEDLINE, Embase, Scopus, Web of Science, Cochrane CENTRAL.  
Grey literature: ProQuest, ClinicalTrials.gov, bioRxiv/medRxiv.  
Hand-searching of reference lists from included studies and reviews.

## Search Strategy

A comprehensive, Boolean-based search will be performed. Full database-specific strategies provided in Appendix 1.

## Study Selection

Titles/abstracts screened by two independent reviewers.  
Full texts assessed for eligibility.  
Discrepancies resolved by discussion or third reviewer.  
PRISMA 2020 flow diagram will be used to report selection.

## Data Extraction

Pre-piloted form (Appendix 2). Two reviewers extract independently. Domains include study characteristics, population, intervention, comparator, outcomes, microbiome methods, key findings, limitations.

## Risk of Bias Assessment

Tools selected according to study design (Appendix 3). Two reviewers independently assess. Results presented narratively and visually.

## Data Synthesis

Narrative synthesis: All included studies will be summarized in structured tables.  
Quantitative synthesis (meta-analysis): Conducted if ≥3 studies with comparable interventions/outcomes.  
- Random-effects model will be default.  
- Subgroup and sensitivity analyses will be conducted (Appendix 4).  
Publication bias: Funnel plots and Egger’s test (≥10 studies).

## Confidence in Cumulative Evidence

GRADE will be applied to rate overall certainty.

# Dissemination

Findings will be submitted to a peer-reviewed journal and presented at conferences.

# Appendices

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## Appendix 1. Search Strategies

A draft PubMed/MEDLINE strategy (to be adapted for Embase, Web of Science, Scopus, and CENTRAL):  
  
((microbiome OR microbiota OR "gut flora" OR "intestinal microbiome" OR "gut microbiota" OR dysbiosis) AND (epigenetics OR transcriptome OR gene regulation OR "gene expression" OR "chromatin remodeling" OR methylation OR acetylation OR transcriptomics OR genomics OR proteomics OR metabolomics) AND (disease OR prevention OR therapy OR health OR cancer OR diabetes OR obesity OR inflammation OR neurodegeneration OR cardiovascular OR mental health))  
  
Filters: Humans, Animals, English. No date restriction.

## Appendix 2. Data Extraction Template

Study ID | Author, Year | Country | Study Design | Population/Model | Intervention | Comparator | Microbiome Assessment Method | Gene Regulation Outcomes | Health Outcomes | Key Findings | Limitations  
  
This table will be piloted and refined prior to extraction.

## Appendix 3. Risk of Bias Assessment Plan

- RCTs: Cochrane Risk of Bias 2 (RoB 2)  
- Non-randomized studies: ROBINS-I  
- Animal studies: SYRCLE tool  
- In vitro mechanistic studies: Adapted NIH Quality Assessment Tool  
  
Two reviewers will independently assess, with consensus or third-party resolution.

## Appendix 4. Planned Data Synthesis & Analysis

- Narrative synthesis structured by intervention type and outcome domain.  
- Meta-analysis (random-effects) if ≥3 studies with comparable interventions and outcomes.  
- Subgroup analyses: Human vs animal vs in vitro, intervention types, outcome domains.  
- Sensitivity analyses: Excluding high risk of bias studies, excluding small sample sizes.  
- Publication bias: Funnel plots + Egger’s regression test (if ≥10 studies).  
- Certainty of evidence: GRADE framework applied per outcome domain.

## Appendix 5. PRISMA-P 2015 Checklist

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| --- | --- | --- |
| Section | Checklist Item | Reported (Yes/No/Page) |
| Title | Identify the report as a protocol of a systematic review |  |
| Registration | If registered, provide registry name and number (e.g., PROSPERO) |  |
| Authors | Provide name, affiliation, and roles of protocol contributors |  |
| Amendments | State plan for documenting protocol amendments |  |
| Support | Describe sources of support, sponsor, and role of funders |  |
| Introduction | Provide rationale for the review in context of what is already known |  |
| Objectives | State explicit objectives with reference to participants, interventions, comparators, and outcomes (PICO) |  |
| Eligibility criteria | Specify study characteristics (PICO, study design, setting, time frame, language) |  |
| Information sources | Describe all intended information sources (databases, registers, etc.) |  |
| Search strategy | Present draft of at least one electronic search strategy |  |
| Data management | Describe mechanisms for managing records and data throughout review |  |
| Selection process | State how studies will be selected (two reviewers, consensus, etc.) |  |
| Data collection process | Describe how data will be extracted from reports |  |
| Data items | List and define all variables for which data will be sought |  |
| Outcomes | List and define all outcomes for which data will be sought |  |
| Risk of bias | Describe anticipated methods for assessing risk of bias in individual studies |  |
| Synthesis | Describe criteria under which data will be synthesized and planned methods |  |
| Meta-bias(es) | Specify any planned assessment of meta-biases (publication bias, selective reporting) |  |
| Confidence in cumulative evidence | Describe how strength of body of evidence will be assessed (e.g., GRADE) |  |