Automating a Systematic Review: Analysis of the Gut Bone Axis

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TRABAJO FINAL DE GRADO BIOTECNOLOGÍA

UNIVERSITAT ROVIRA i VIRGILI

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Tarragona, 2024

Data de convocatòria: Setembre 2024

Jo, Tomás Cantón Cordeiro, amb DNI 44498445T, sóc coneixedor de la guia de prevenció del plagi a la URV Prevenció, detecció i tractament del plagi en la docència: guia per a estudiants (aprovada el juliol 2017). (http://www.urv.cat/ca/vidacampus/serveis/crai/queus-oferim/formaciocompetencies-nuclears/plagi/) i afirmo que aquest TFG no constitueixen cap de les conductes considerades com a plagi per la URV.

Tarragona, 25 de agost de 2024

Contents

List of Figures

List of Tables

1 Collaboration and research center data

This final degree project has been carried out in collaboration with the Medicity Research Laboratory at the University of Turku, Finland, and the Phylobone research group.

The Medicity Research Laboratory is a research center dedicated to advanced biomedicine and translational medicine. This laboratory hosts around 130 scientists and technicians and is known for its focus on cells, tissues, and living organisms. Medicity has implemented cutting-edge technologies, such as multiphoton microscopy and PET imaging systems for small animals. The research projects range from basic studies to clinical initiatives, and the laboratory has gained recognition both nationally and internationally.

The Phylobone research group, led by Dr. Miho Nakamura and Dr. Pere Puigbò, specializes in bone regeneration through the development of bio-inspired biomaterials. The group's main objective is to prevent and treat bone diseases such as osteoporosis and osteoarthritis. The group's research integrates medicine, cell biology, and materials science to develop new therapeutic strategies.

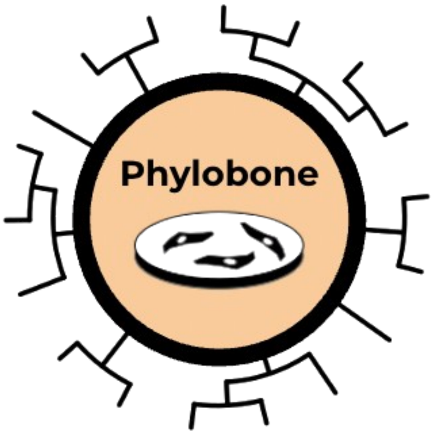

Figure 1.1. Phylobone research group logo.

2 Abstract

The study focuses on analyzing the gut-bone axis, exploring how intestinal microbiota and metabolites influence bone health. The growing concern about osteoporosis, a disease that affects bone density and increases the risk of fractures, underscores the need to develop new therapeutic strategies. The hypothesis is that microorganisms in the gut microbiome can regulate bone formation through the gut-bone axis, with the objective of identifying these interactions through a comprehensive review of scientific literature.

The methodology employed includes a systematic review based on the PubMed database, complemented by the PRISMA methodology to guide the study selection. Boolean search strategies and specific inclusion and exclusion criteria were applied to filter relevant studies. The selection of articles was optimized through the generation of specific keywords and the use of automation tools, including algorithms and iterative filtering techniques, facilitating a precise and efficient compilation of information.

The results include the creation of a detailed database that provides a comprehensive view of interactions within the gut-bone axis, highlighting specific examples such as the *Lactobacillus rhamnosus GG* (LGG) strain. This strain plays a key role in modulating metabolic pathways, such as the pentose and glucuronate interconversion pathway (map00040), where it regulates the activity of the enzyme beta-D-glucuronidase, directly impacting in estrogen homeostasis, which is crucial for bone health in postmenopausal women.

In conclusion, the study confirms the importance of the gut-bone axis in bone health and suggests that modulating the intestinal microbiota could be a promising strategy to prevent and treat bone diseases such as osteoporosis. Furthermore, the effectiveness of the automated techniques can accelerate the research and contribute to the development of new therapeutic interventions.

Keywords

Bone formation, Bone resorption, Gut-brain-bone axis, Intestinal microbiota, Metabolic pathways, Osteoporosis, PRISMA, Probiotics

Key abbreviations

LGG: *Lactobacillus rhamnosus GG*; **LPS**: Lipopolysaccharide; **PRISMA**: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; **SCFA**: Short-Chain Fatty Acids; **TMAO**: Trimethylamine N-oxide; **ECM**: Extracellular Matrix; **AI**: Artificial Intelligence; **BMD**: Bone Mineral Density

3 Introduction

3.1 Osteoporosis

Osteoporosis is a metabolic bone disease characterized by decreased bone mass and deterioration of bone microarchitecture, leading to increased bone fragility and a higher risk of fractures. The World Health Organization (WHO) defines it as a progressive systemic skeletal disease marked by low bone mass and structural deterioration of bone tissue. This condition predominantly affects individuals over the age of 60, with an estimated 200 million people affected globally. Osteoporotic fractures are a major health concern, significantly impacting quality of life and contributing to morbidity and mortality, particularly due to hip, spine, and wrist fractures [1].

The primary cause of osteoporosis is an imbalance between bone resorption and bone formation, where bone resorption exceeds bone formation, leading to weakened bones. Osteoporotic fractures occur frequently, with an estimated 8.9 million fractures annually. The risk of fracture increases as bone mineral density (BMD) decreases [1].

The bone extracellular matrix (ECM) contains minerals deposited on highly crosslinked collagen fibrils and hundreds of non-collagenous proteins. Some of these proteins are key to the regulation of bone formation and regeneration via signaling pathways, playing important regulatory and structural roles. The process of osteogenesis, involving the differentiation of mesenchymal stem cells into osteoblasts that produce the bone ECM and regulates mineralization, is vital for maintaining bone health and strength [2].

Given the rising global life expectancy, the prevalence and impact of osteoporosis are expected to grow, needing effective management strategies. As a result, new therapeutic approaches are being explored to improve treatment efficacy and safety [1].

3.2 Gut bone axis

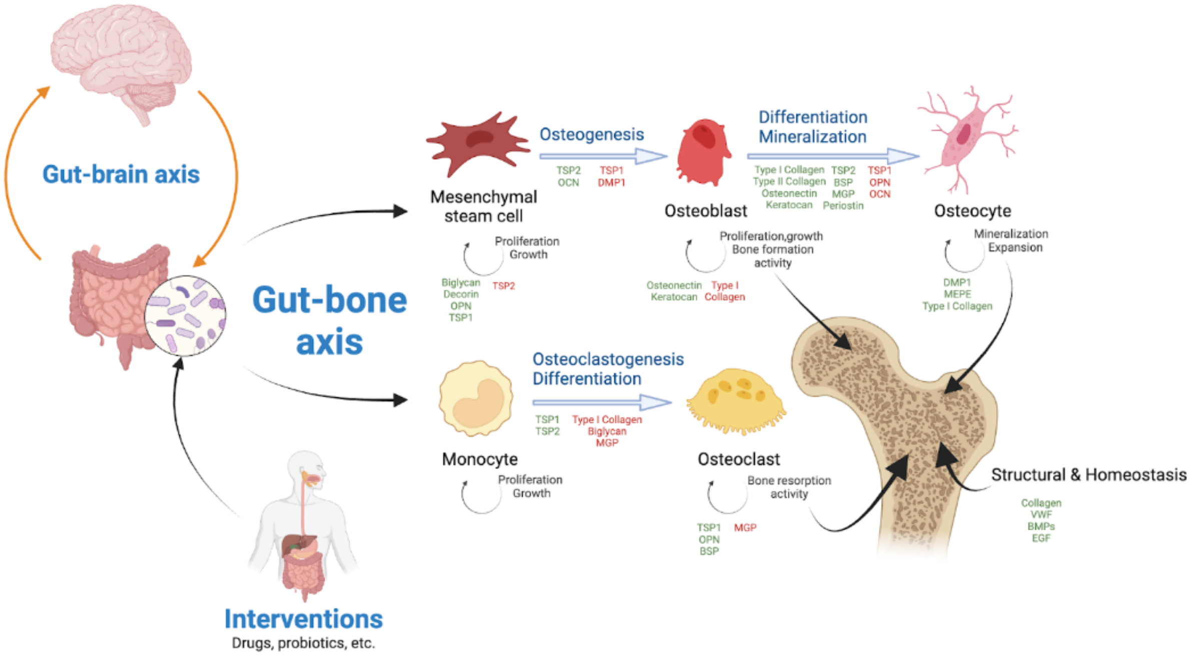

Figure 3.1. Illustration of the gut-brain-bone axis and its role in bone health. Modified from [2] http://creativecommons.org/licenses/by/4.0/

The gut-bone axis influences bone health through various pathways that are critical for bone formation and maintenance, including osteogenesis, osteoclastogenesis, and mineralization. Osteogenesis involves the differentiation of mesenchymal stem cells into osteoblasts, which are responsible for bone formation. Conversely, osteoclastogenesis refers to the differentiation of monocytes into osteoclasts, which are essential for bone resorption. This process is crucial for bone remodeling and the maintenance of bone homeostasis. Both osteogenesis and osteoclastogenesis are regulated by several key proteins, including TSP1, TSP2, OPN, and MGP, which play vital roles in these processes.

The gut-brain axis connects the central nervous system with the gastrointestinal tract, influencing both brain functions and bone health through various signaling mechanisms. Neurotransmitters and hormones produced in the gut can significantly affect bone density and quality by modulating the activity of osteoblasts and osteoclasts.

Figure 3.1 also highlights the role of probiotics and drugs in modulating these axes to improve bone health. Probiotics, in particular, can influence the production of metabolites, which possess various properties that enhance bone formation by promoting osteoblast activity and inhibiting osteoclastogenesis.

3.3 Phylobone project

Phylobone [2] is a comprehensive database designed to catalog ECM proteins in bone from humans and various model organisms. This resource includes information on 255 proteins potentially expressed in the bone ECM of humans and 30 vertebrate species. The database integrates protein sequences, functional characterizations, and potential drug interactions, facilitating research in bone regeneration or osteoporosis. The primary objective of Phylobone is to enhance our understanding of bone ECM proteins and their roles in bone formation and resorption. By employing a bioinformatics pipeline, Phylobone identifies these evolutionary relationships, which aids in pointing to potential model organisms for studying bone regeneration. A network analysis revealed high connectivity among bone ECM proteins, identifying a total of 214 functional protein domains, including those involved in bone formation and resorption. The database also offers insights into drug repurposing opportunities by linking existing drugs to bone ECM proteins, potentially accelerating the development of new treatments for osteoporosis and other bone disorders. Phylobone provides a robust platform to study bone regeneration and osteoporosis in light of biological evolution, substantially contributing to the identification of molecular mechanisms and drug targets.

3.4 PRISMA

In this thesis, a systematic review—a rigorous and transparent method used to identify, evaluate, and synthesize studies—will be conducted to explore the relations between the gut-bone axis and how intestinal bacteria and metabolites influence bone health [3].

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method will be applied to ensure the systematic review is comprehensive and transparent. PRISMA is a set of guidelines designed to help researchers report systematic reviews and meta-analyses clearly and transparently, improving the quality of these studies [4]. The next steps will be followed in the PRISMA method:

1. Formulate the review question
2. Define inclusion and exclusion criteria
3. Develop a search strategy and locate studies
4. Select studies
5. Extract data
6. Assess study quality
7. Analyze and interpret results
8. Disseminate findings

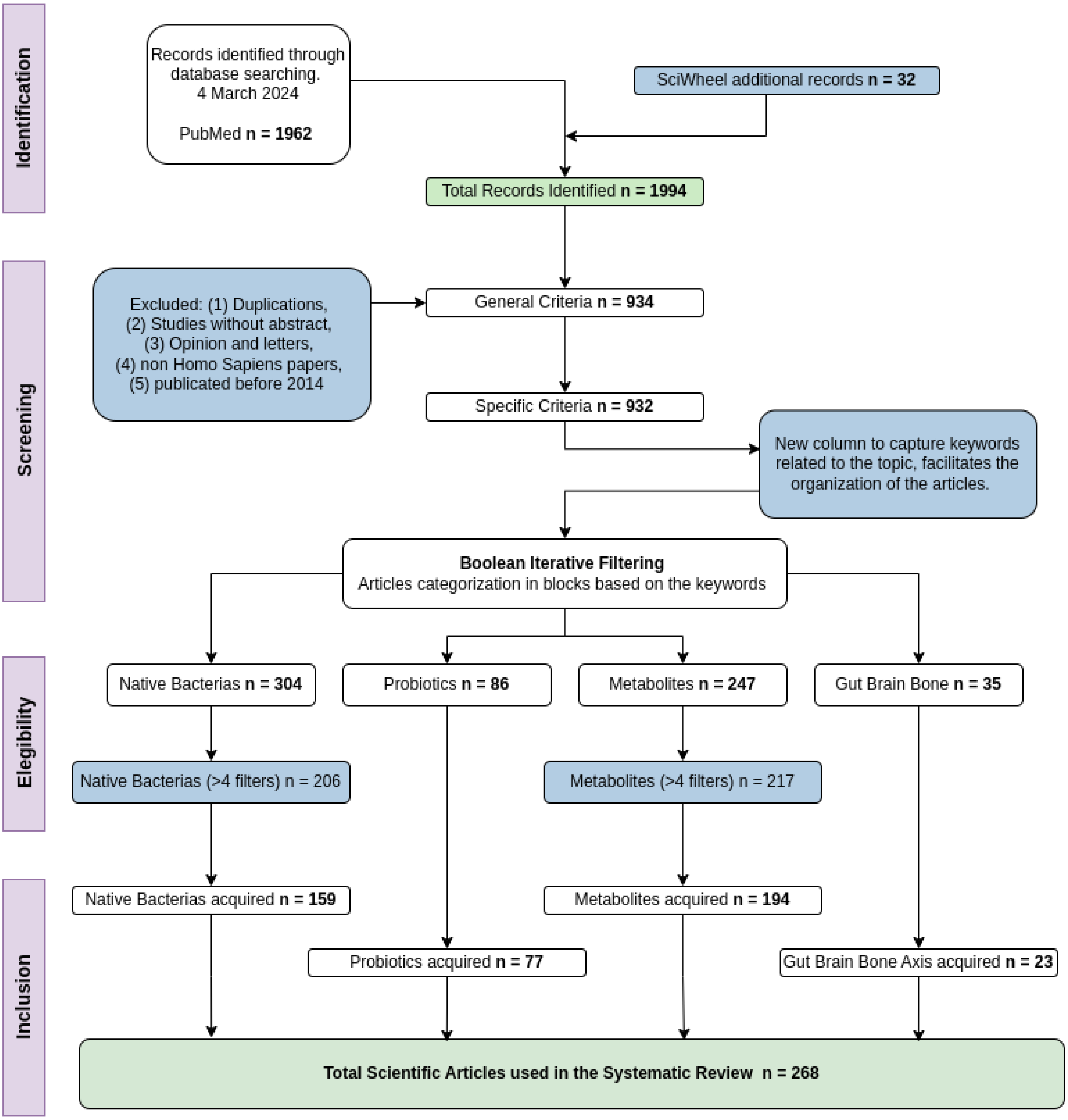

Figure 3.2. PRISMA flow diagram. This diagram visualizes the key steps in the systematic review, from the identification of articles to final inclusion of 268 relevant studies.

4 Hypothesis and Objectives

The bidirectional interaction between the gut and bones is mediated by various metabolic, hormonal, and immunological factors. This interaction includes the influence of gut microbiota and metabolites that play a role in bone regeneration and maintenance of bone health, helping to prevent diseases such as osteoporosis.

Our hypothesis is that microbes from the gut microbiome may play a regulatory role in stimulating or inhibiting bone formation and regeneration through the gut-bone axis. In this study, a comprehensive review of the scientific literature will be conducted to identify the interactions between gut microorganisms and the molecules involved in the process of bone formation and regeneration.

Several objectives will be addressed throughout this thesis. First, the role of metabolites and microbiota in the gut-bone axis will be evaluated, identifying the probiotics and metabolites involved and how they influence bone health. Second, the metabolic pathways involved in the brain-gut-bone axis will be investigated, examining how probiotics and metabolites interact within these pathways and contribute to the balance between these systems. Third, the Phylobone database will be compared with the data obtained from this review, analyzing which proteins and probiotics are the most frequent. Finally, the study will verify if the technology used and the automation processes implemented in the different steps of the systematic review can speed up the research and help identifying potential therapies or interventions in the biomedical field.

5 Methodology

5.1 Databases and Search strategies

The search and selection of information for this systematic review were conducted through PubMed, a comprehensive database of scientific abstracts and medical citations, managed by the National Center for Biotechnology Information (NCBI) within the National Library of Medicine and the National Institutes of Health (NIH). PubMed provides access to a wide range of life sciences databases, containing over 3 billion records, which facilitates an exhaustive search of relevant scientific articles for any research topic [5].

Each article selected from PubMed undergoes a peer-review process, which is a standard practice ensuring the quality and relevance of the studies included in the database. This procedure adds an additional level of reliability to the articles chosen for the systematic review, guaranteeing a quality selection for the study topic [5].

The search strategy designed for this study was based on Boolean search terms to capture as much relevant literature as possible regarding the gut-bone axis. The search terms used were:

(microbiota [Title/Abstract] OR microbiome [Title/Abstract]) AND (Bone [Title/Abstract])

Query 1. Search performed in PubMED to identify articles.

This query was chosen with the aim of identifying studies discussing the relationship between the microbiota and bone health in humans. The search strategy was developed in consensus with the Phylobone research group, ensuring that it accurately captured the scope of relevant literature for this systematic review. The search initially yielded a total of 1962 articles at March 4, 2024.

For the processing and management of the collected information, Sciwheel, a bibliographic management platform, was used. Sciwheel facilitated the review and suggestion of additional studies, resulting in 32 articles suggested by the tool, which were added to the dataset, obtaining a total of 1994 articles. With the support of Sciwheel, all articles were downloaded in BIB format (see A), which facilitated the organization and portability throughout the systematic review.

5.1.1 Generation of Keywords

To facilitate data manipulation, a preprocessing was performed using Python and libraries like Pandas. The BIB file was transformed into a Comma-Separated Values (CSV) file as it offers advantages such as ease of reading and writing, greater compatibility with different data analysis tools, and simplicity in structuring information in tables.

Prompts were generated for each article based on its title and abstract. These prompts were sent to an Artificial Intelligence (AI) tasked with generating specific keywords. Using a Python script, the process of sending each article to the AI was automated. For each article, the title and abstract were dynamically substituted into the general prompt, which was then sent to the AI for keyword generation. The generated keywords for each article were added as a new column in the CSV file. These keywords summarized the article's information concisely and accurately. These generated keywords will be the basis for applying inclusion and exclusion criteria to refine the dataset.

5.2 Inclusion and exclusion criteria

5.2.1 General criteria

To ensure the relevance and quality of the information included in the systematic review, general inclusion and exclusion criteria were established. These criteria were applied to filter the initial database obtained from the PubMed search.

The following studies were excluded: (1) articles that did not have an abstract, since it is essential for the preliminary evaluation and for the generation of keywords that will be used later; (2) studies such as opinion articles or letters, as they do not provide relevant data for the research; (3) non Homo Sapiens studies, as the purpose of this review is to directly evaluate the interaction between human microbiome and bone health;(4) the articles must contain the word 'gut' for relate all the articles in the context of the gut-bone axis; (5) the articles were limited to studies published in the last ten years, from 2013 to 2024.

The application of these general inclusion and exclusion criteria allowed the optimization of the selection, facilitating the focus of efforts on the most relevant studies for the research objectives. As a result of applying these general criteria, the total number of articles was reduced to 932. This set of articles advanced to the next phase of the review, where specific inclusion and exclusion criteria were applied to buid the data tables proposed in this research.

5.2.2 Specific criteria

To ensure the relevance of the selected articles, a list of keywords related to bone health and the gut was established. This list includes microorganisms and probiotics, biological processes such as osteogenesis and osteoclastogenesis, also components such as proteins, peptides or metabolic pathways were included. The query was designed to fulfill these terms, allowing the filtering of articles that contain information directly relevant to the research objective. The keywords presented here serve as an illustrative set; the full list used in the study is more extensive and detailed.

Once the query was defined, a Python algorithm was developed tp compare the keywords column generated for each article with the query's list of keywords. This algorithm acted as a filter, identifying articles that contained relevant terms. The final result was the creation of a new column for each article called *Queried*, which contains the keywords of interest present in each study.

Table 5.1. Keyword list for comparative analysis of gut-brain-bone axis.

- Absorption
- Actinomyces
- Akkermansia
- Amyloid
- Anaerofustis
- Anaerotruncus
- Axis
- Bacteroides
- BMP
- Bone
- Bone formation
- Bone generation
- Bone health
- Bone resorption
- Brain
- Citrobacter
- Clostridium
- Collinsella
- Collagen
- Cytokine
- Degradation
- Desulfovibrio
- Drug
- EGF
- ECM
- Elastase
- Enterobacter
- Escherichia
- Eubacterium
- Extracellular
- Fermented
- Fibro
- Fusobacterium
- Gut
- Gut-bone axis
- Gut-brain-bone
- Holdemania
- Hormone
- Immune system
- Indigenous
- Inflammation
- Inhibitor
- Integrin
- Intestinal
- Intrinsic
- Microbiome
- Microbiota
- Mollicutes
- Molecule
- Native
- Neuro
- Nervous system
- Osteoblast
- Osteoclast
- Osteogenesis
- Pathway
- Peptide
- Prebiotic
- Metabolite
- Metabolism
- Metabolic
- Peptide
- Prebiotic
- Protein
- Protease
- Probiotic
- Saccharomyces
- SCFA
- Serine
- Signal
- Stem
- Stem cells
- Streptococcus
- Supplement

5.2.3 Iterative filtering for classification

To achieve a more precise selection of the studies that will contribute to the construction of the four proposed tables, a iterative filter were implemented. These boolean filter was executed through a Python script. The code facilitated the creation of detailed queries with keywords related to the specific theme of each table, using boolean operators as 'AND' to restrict and 'OR' to include or classify the articles according to the keywords.

The 'AND' operators were used as restrictive filters, meaning that the articles must contain the keywords to be included. On the other side, the 'OR' operators were used as classificatory filters, if the articles contain the words, they will be added to the correspoding column, if they do not contain the 'OR' keywords, they will still be included as long as they meet with the 'AND' criteria.

These filters (see Appendix A) were used to capture the maximum number of relevant terms, ensuring that each table contains specific and pertinent studies for the research objectives. These examples demonstrate the initial filtering process, but additional 'OR' filters were subsequently applied to comprehensively obtain the maximum number of articles belonging to each table. This iterative filtering approach ensures a thorough and targeted selection of articles for each category.

5.3 Data extraction

To analyze the studies and compile the relevant data for each of the four proposed tables, an iterative approach was used to prioritize the articles based on the number of keywords matched. The articles that contained the highest number of specific keywords were given higher priority and positioned at the top of the list for further analysis. This score ensured that the most relevant studies were reviewed first, maximizing the quality and relevance of the extracted data.

Efforts were made to obtain the full-text PDFs of the best articles. Access issues, such as institutional restrictions or private publications, were documented, and the corresponding articles were excluded from further analysis.

Table 5.2. Summary of articles analyzed. For native bacteria and metabolic pathways, articles with more than 4 keyword matches were prioritized.

Category	**Total Articles**	**Keyword Matches**	**Articles Obtained**
Native bacteria	304	206 ($x > 4$)	159
Probiotic strains	86	86	77
Metabolic pathways	247	217 ($x > 4$)	194
Gut-brain-bone interactions	35	35	23

Once the relevant articles were obtained, an AI was employed to automate the extraction of the information. Each article was processed by attaching it to a specific prompt for each category. Native bacteria related to the gut-bone axis, probiotic strains, metabolites and metabolic pathways, or gut-brain-bone interactions. The AI system scanned the entire text of the article, extracting all relevant information in a narrative way.

After the AI summarized the information, the extracted data were organized into columns corresponding to each article. Depending on the category, these columns could include details or other relevant points. This information was then systematically added to a growing database, ensuring that all details were captured in a structured format.

The automation of this process allowed for the efficient and accurate compilation of a database that captured the complexity of the gut-bone axis interactions.

5.4 Quality control

To ensure the accuracy and reliability of the results obtained in this study, a quality control was conducted. This process included the classification of articles depending to the number of keywords used in the iterative filtering that each article contained. Articles with the highest number of keywords were considered the most aligned with the criteria of the systematic review. To verify the accuracy of the results, the top 5 highest-scoring articles in each category were manually selected and analyzed, reaching a total of 20 articles reviewed.

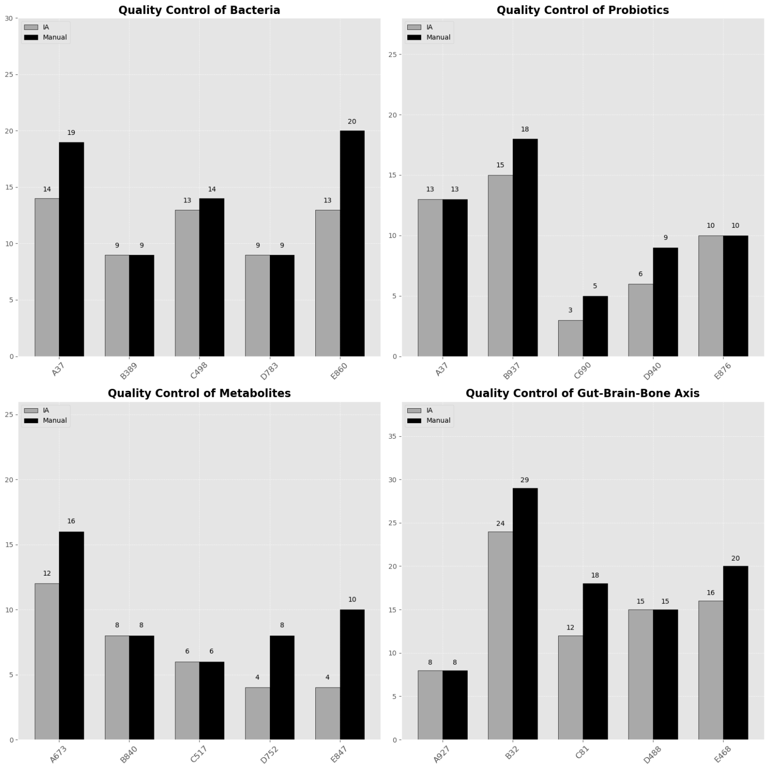

Figure 5.1. Comparison of results obtained by AI and manual review.

For articles with fewer than 10 pages, the data extraction was nearly 100%, indicating an excellent performance in short documents. However, for articles with more than 15 pages, a loss of results was observed, especially towards data extracted located in the final pages. The manual review also revealed that the AI is more efficient at extracting data from tables than from figures or images. The AI processes text in tables better; extracting text from images presents greater challenges due to different format and quality.

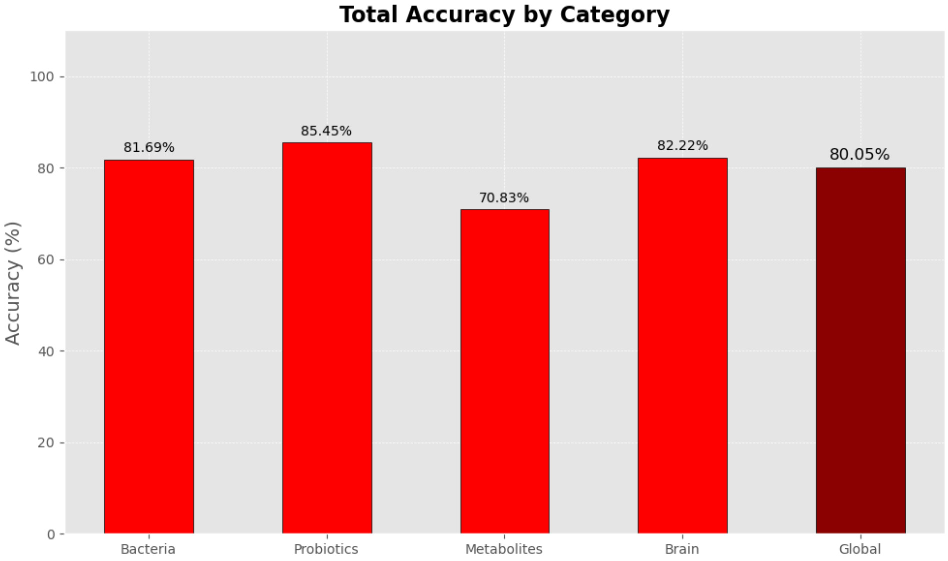

Figure 5.2. Total accuracy percentage in each category of tables.

The categories of bacteria and probiotics showed a higher accuracy rate compared to the category of metabolites. A possible explanation is that the scientific names of bacteria are often italicized, which might make it easier for the AI to identify text with special formatting.

The overall accuracy rate is around 80%, indicating high precision and validating the method used for data extraction in this systematic review.

6 Results

6.1 Gut - bone axis

The gut microbiota plays a crucial role in bone health, influencing the formation and resorption of bone through a complex interactions of microbial metabolites, immune modulation, and hormonal regulation. These interactions can be positive or negative, depending on the specific bacteria and metabolites involved. The gut-bone axis highlights the importance of having a microbial balance for maintaining skeletal health, with some native bacteria and their metabolites promoting osteogenesis, while others driving to osteoclastogenesis and bone resorption. The following results explore the role of native gut bacteria and the metabolites they produce in regulating these critical processes.

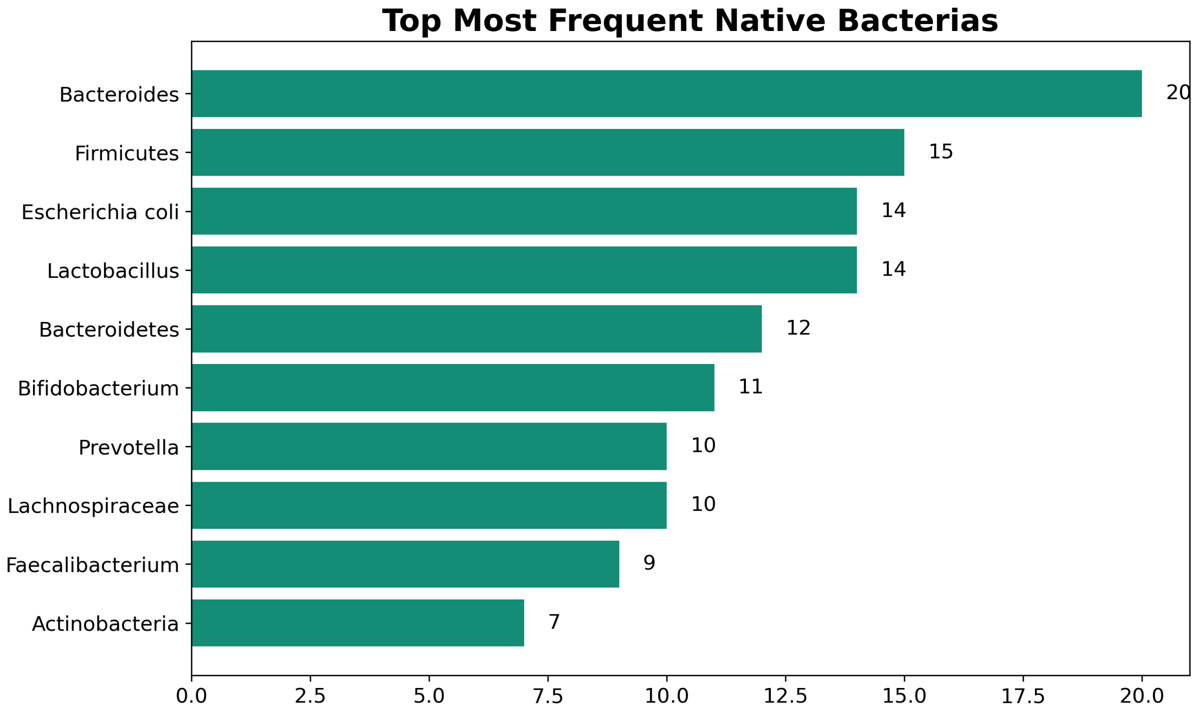

Figure 6.1. Top most frequent native bacteria. (The results are superficial and do not provide highly specific information.)

Native bacteria supporting bone formation

Several gut bacteria play a significant role in promoting osteogenesis, primarily through the production of short-chain fatty acids (SCFAs) and modulation of the immune system. *Lactobacillus acidophilus*, for example, promotes bone formation by balancing the Treg-Th17 cell axis, which reduces inflammation and supports osteoblast activity, crucial for bone health. Similarly, *Bifidobacterium longum* enhances BMD by increasing the bioavailability of polyphenols and producing butyrate, a potent anti-inflammatory SCFA that supports bone health [13]. The *Lachnospiraceae* family, including *Eubacterium* and *Roseburia*, also contribute significantly to osteogenesis through butyrate production, which enhances osteogenic activity and reduces bone resorption. Additionally, *Faecalibacterium prausnitzii* is a major butyrate producer, known for its anti-inflammatory properties, which positively impact bone health by promoting bone formation. Actinobacteria, particularly those within the genus *Bifidobacterium*, support bone health through SCFA production, especially butyrate, which modulates immune responses and promotes bone formation [31].

Native bacteria promoting bone resorption

In contrast, certain gut bacteria are associated with promoting osteoclastogenesis and bone resorption, often through inflammatory pathways or the production of harmful metabolites. *Escherichia coli*, particularly pathogenic strains, are implicated in the production of trimethylamine N-oxide (TMAO), a metabolite strongly associated with increased bone resorption and the progression of osteoporosis. Additionally, *Bacteroides fragilis* and other species within the genus are more abundant in individuals with osteoporosis, suggesting a role in promoting bone resorption, although some strains like *Bacteroides ovatus* have been linked to bone strength in different contexts [26]. *Prevotella copri* is particularly noted for its association with increased severity of rheumatoid arthritis, a condition that leads to significant bone erosion and highlights the potential for *Prevotella* spp. to negatively impact bone health through systemic inflammation [21]. *Clostridium difficile* is another bacterium linked to gut dysbiosis and systemic inflammation, both of which can negatively affect bone health, although the genus also includes species like *Clostridium butyricum*, which can support osteogenesis under certain conditions. Finally, while Firmicutes generally support gut health, specific species within this phylum may have negative implications for bone health in certain inflammatory conditions, showcasing the dual nature of their impact depending on the context.

6.1.1 Metabolites

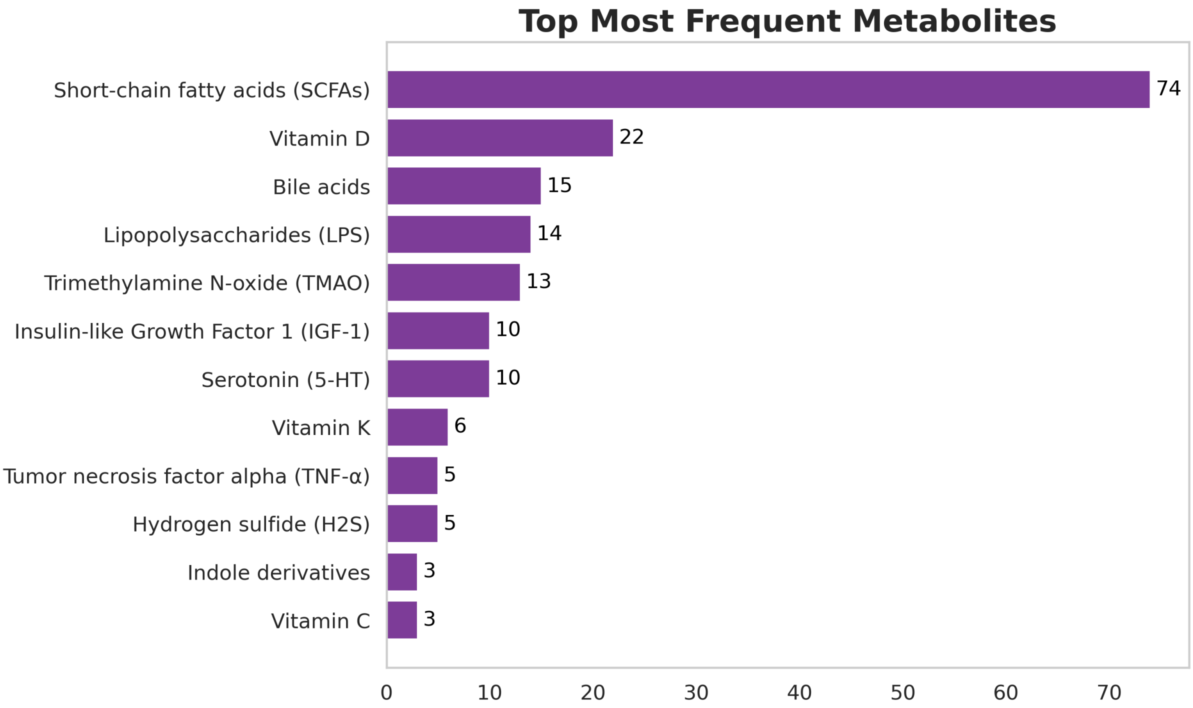

Figure 6.2. Top most frequent metabolites. SCFAs, are the most frequent identified.

The most relevant metabolites are grouped and described based on their categories, main metabolic pathways, and their impact on bone health.

SCFAs

SCFAs, including butyrate, propionate, and acetate, are products of the fermentation of complex carbohydrates by the gut microbiota. These SCFAs activate the Wnt/β-catenin pathway, promoting the differentiation of mesenchymal stem cells into osteoblasts, thereby enhancing bone formation. Additionally, butyrate reduces systemic inflammation by inhibiting NF-κB signaling, protecting against bone loss [34].

Vitamins and minerals

Various micronutrients regulated by the gut microbiota or influenced by gut health play a significant role in bone health. Vitamin D is fundamental for calcium absorption and bone mineralization, with its activation and metabolism closely linked to intestinal function. Calcium absorption in the intestine is crucial for maintaining bone density and is directly dependent on adequate levels of vitamin D. Other micronutrients, such as selenium and vitamin C [33], contribute to bone health primarily through their antioxidant effects and their influence on collagen synthesis, which is vital for maintaining bone structure and strength.

Amino acid-derived metabolites

Some metabolites derived from the metabolism of amino acids, such as tryptophan and phenylalanine, have a notable impact on bone health. Picolinic acid (PIC), a product of tryptophan catabolism, has anabolic effects that promote bone formation. Similarly, indoles, also derived from tryptophan, modulate the immune system and possess anti-inflammatory properties, which help protect against bone loss by reducing systemic inflammation [17].

Hormones and growth factors

The interaction between the gut and the regulation of hormones and growth factors significantly influences bone health. Melatonin, with its antioxidant and anti-inflammatory effects, positively impacts bone formation by activating pathways such as Wnt/β-catenin, which are essential for osteoblast differentiation and function [32]. Insulin-like growth factor 1 (IGF-1) serves as a critical connection between gut health and bone density, influencing bone growth and remodeling through its regulatory effects on bone formation [31].

Inflammatory and toxic metabolites

Some metabolites can negatively affect bone health, particularly those that induce inflammation. Lipopolysaccharides (LPS), which act as bacterial endotoxins, contribute to bone loss by promoting systemic inflammation, a process detrimental to bone remodeling and integrity [8]. TMAO, produced by the gut microbiota from L-carnitine, is associated with increased bone loss, especially in weight loss contexts, due to its pro-inflammatory effects [31].

6.1.2 Probiotics

The figure 6.3 shows the frequency of appearance of different probiotic species in the analyzed studies. The relevance of *Lactobacillus reuteri* is remarkable, since it appears as the most mentioned species, with 24 occurrences. *Lactobacillus rhamnosus* and *Lactobacillus casei* are the following ones, having potential in improving bone health through mechanisms such as calcium absorption. Other species like *Lactobacillus acidophilus* and *Lactobacillus plantarum* also have presence, contributing to the promotion of osteogenesis and the maintenance of bone homeostasis.

The results show the importance of *Lactobacillus* and *Bifidobacterium* species in promoting bone health through different metabolic pathways and the production of specific metabolites.

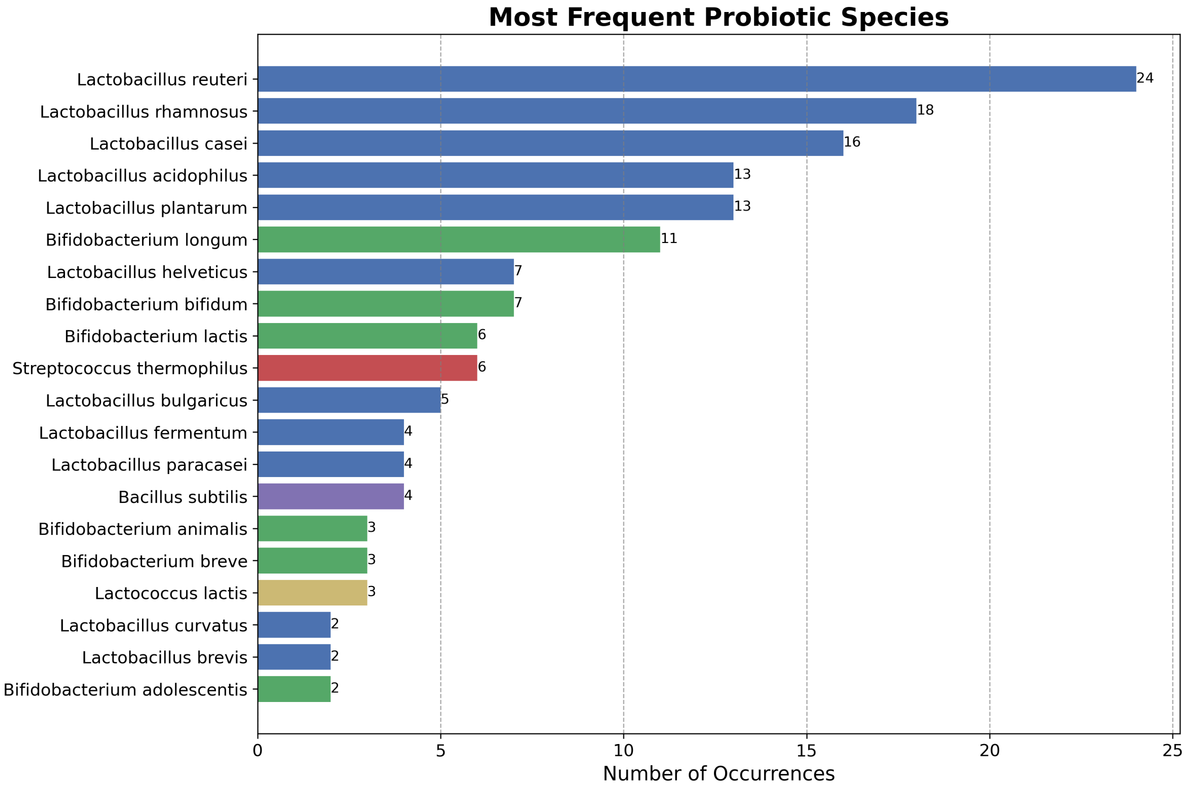

Figure 6.3. Most frequent probiotics (Genus and Species). This figure shows the most frequently identified probiotic species. **Lactobacillus** (blue), **Bifidobacterium** (green), **Streptococcus** (red), and **Bacillus** (purple).

The table 6.1 presents a summary of the most found probiotic strains and their effects on bone health. The full table with all the probiotic results extracted and the rest of the datasets from the articles are found in the database (Appendix A.1).

Among the most frequently mentioned probiotic strains in the studies there are:

Lactobacillus rhamnosus GG is the most frequently mentioned probiotic. It is known for improving gut health, preventing TNF-α mucosal damage, and enhancing calcium absorption. LGG will be studied in more detail later.

Lactobacillus reuteri ATCC 6475 is notable for its ability to improve BMD in postmenopausal women. This strain suppresses the expression of pro-inflammatory and pro-osteoclastogenic cytokines, reducing bone resorption and contributing to immune system modulation.

Bacillus subtilis C-3102 stands out for its ability to increase BMD, inhibit bone resorption, and improve calcium absorption. This probiotic supports osteoblast proliferation and differentiation through butyrate production.

Lactobacillus casei Shirota participates in the production of low molecular weight metabolites that contribute to anti-inflammatory effects, which is crucial for fracture healing and reducing markers of osteoarthritis.

Table 6.1. Summary of probiotic strains and their effects on bone health

Probiotic Strain	**Effect on Bone Health**	**Metabolic Pathways and Metabolites**
Lactobacillus rhamnosus GG	Improves gut barrier health, prevents TNF-α induced mucosal damage, enhances calcium absorption.	Enhances mucin expression, produces SCFAs increasing calcium solubility and absorption. Protein HM0539 involved.
Lactobacillus casei Shirota [21, 18]	Enhances gut health, reduces inflammation, improves fracture healing, reduces markers of osteoarthritis.	Produces low molecular weight metabolites, increases IL-10, reduces TNF-α, IL-6, and IL-12, aiding in anti-inflammatory effects.
Bacillus subtilis C-3102 [18, 28, 27]	Increases BMD, inhibits bone resorption, improves calcium absorption.	Increases uNTx and TRACP-5b, enhances gut microbiota, increases butyrate production which supports osteoblast proliferation and differentiation.
Lactobacillus reuteri ATCCPTA 6475 [28]	Reduces BMD loss in older women.	Modulates gut microbiota, suppresses the expression of pro-inflammatory and pro-osteoclastogenic cytokines reducing bone resorption.
Lactobacillus plantarum GKM3 and DSM 15312 [28]	Inhibits bone loss, promotes osteogenesis, decreases osteoclastogenesis.	Produces SCFAs, regulates VDR and TRPV6 for calcium absorption, modulates claudin-2 for intestinal permeability.
Lactobacillus paracasei DSM 13434 [28]	Protects against rapid BMD loss in postmenopausal women.	Produces SCFAs, reduces intestinal permeability, decreases pro-inflammatory cytokines like TNF-α.
Lactobacillus intestinalis YT2 [27]	Alleviates menopausal symptoms, including bone density loss.	Restores gut microbiota composition, enhancing overall gut health and indirectly supporting bone health.
Lactobacillus plantarum NK3 [15]	Alleviates bacterial vaginosis and osteoporosis.	Suppresses NF-κB/TNF-α pathway, reducing inflammation and promoting bone health.
Lactobacillus reuteri NCIMB 30242 [15]	Increases serum vitamin D levels, crucial for bone health.	Increases circulating 25-hydroxyvitamin D, supporting bone mineralization and health.
Bifidobacterium lactis Probio-M8 [16]	Improves bone metabolism, increases vitamin D3, decreases PTH and procalcitonin, enhances calcium absorption.	Involves carbohydrate metabolism pathways, enhances gut microbial interactions, increases SCFA-producing bacteria and choline-phosphate cytidylyltransferase.
Streptococcus salivarius W24 [27]	Inhibits periodontopathogens, maintains immune homeostasis.	Targets IL-6 and IL-8 pathways, producing bacteriocins that suppress pathogen growth.
Tenericutes ML615J-28, 124-7 [27]	Reduction in abundance in PCOS patients, potentially improves bone health.	Specific metabolic pathways not clearly defined, but reduction in abundance correlates with improved bone health.
Bifidobacterium lactis HN019 [19]	Reduces IL-1β, RANKL-OPG ratio, TNF-α, and IL-6, disrupts Gram-negative bacteria membrane, reduces *P. gingivalis* adhesion.	Regulates immune system through organic acids like lactic acid, contributing to anti-inflammatory effects.

6.2 Impact of LGG

The table provides a detailed analysis of how LGG's influence on metabolic pathways related to bone health,including SCFA production, the Wnt/β-catenin pathway, and immune modulation. Essential pathways for maintaining bone density and preventing diseases. Furthermore, LGG's enhances vitamin D absorption and reduces inflammation through the inhibition of NF-κB highlighting its potential as a probiotic therapy.

Specifically, LGG has been identified to participate in the pentose and glucuronate interconversion metabolic pathway, according to the KEGG database (pathway map00040). Within this pathway, the enzyme beta-D-glucuronidase plays a crucial role in the metabolism of glucuronides. LGG modulates the activity of this enzyme, affecting the availability of glucuronides, which are important for the excretion of metabolites and the regulation of estrogen levels in the body.

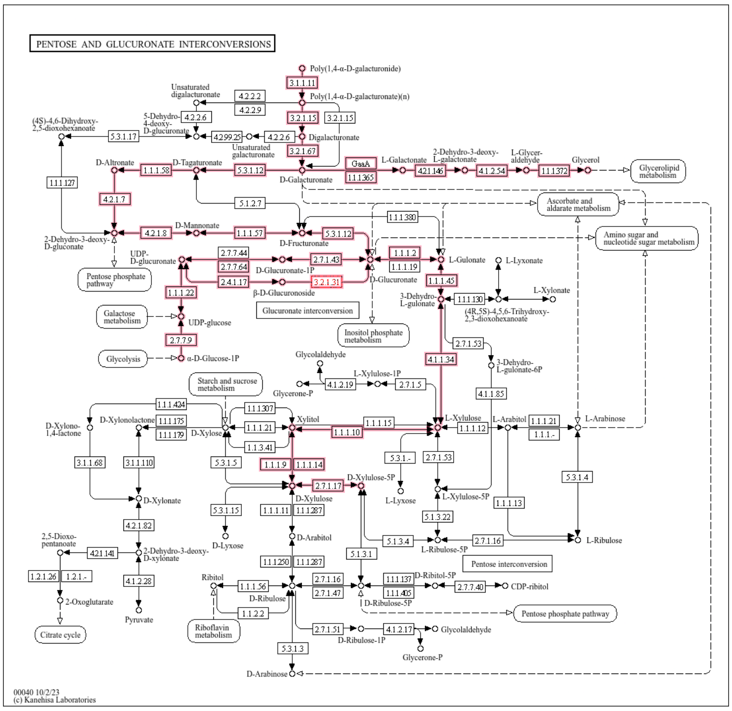

Figure 6.4. Pentose and glucuronate interconversions pathway (map00040)

The modulation of beta-D-glucuronidase by LGG has direct implications for bone health, particularly in maintaining adequate estrogen levels in postmenopausal women, which is essential for preventing osteoporosis. The KEGG database has been a key in mapping these metabolic interactions, facilitating the visualization of how LGG impacts bone homeostasis through its influence on glucuronide metabolism. [39]

This systematic documentation of pathways and metabolites is essential for developing a comprehensive database on the relationship between the gut microbiome and bone health. The information compiled in the table can be valuable for future studies, serving as a guide in the research and helping to identify new areas of study in this complex axis.

Table 6.2. Summary of metabolic pathways, metabolites, and effects of LGG on bone health

Metabolic Pathways and Involved Metabolites	**Effects on Bone Health**	**How LGG Influences**
Production of SCFAs (Butyrate, propionate, acetate) [13, 14, 16, 15, 17, 20]	Promotes the differentiation of mesenchymal stem cells into osteoblasts, improves bone formation, and reduces bone resorption.	Increases the production of SCFAs, especially butyrate, which activates the Wnt/β-catenin pathway and reduces inflammation by inhibiting NF-κB.
Wnt/β-catenin (Butyrate) [13, 16]	Stimulates the accumulation of β-catenin, promoting the proliferation and differentiation of stem cells into osteoblasts.	Increases the production of butyrate, which activates the Wnt/β-catenin pathway to promote bone formation.
NF-κB (Butyrate) [13, 16, 15]	Reduces inflammation, creating a favorable environment for bone formation by inhibiting osteoclast activation.	Producing butyrate, inhibits NF-κB signaling, which decreases inflammation and protects bone mass.
β-D-glucuronidase (Estrogens) [13, 14, 16]	Regulates estrogen levels, essential for maintaining bone density in postmenopausal women.	Reduces β-D-glucuronidase activity, limiting estrogen reabsorption and reducing the risk of reproductive cancers, maintaining a healthy estrogen balance.
Tryptophan Metabolism (Indoles) [17]	Modulates the immune system, decreases inflammation, and supports bone health.	Metabolizes tryptophan to produce indoles, which have anti-inflammatory effects and contribute to beneficial immune modulation for bone health.
Glycolysis (Lactate) [17])	Maintains a healthy intestinal environment that indirectly supports bone health.	Produces lactate through glycolysis, helping maintain an appropriate intestinal pH, favoring the presence of beneficial bacteria that support bone health.
Vitamin D Absorption (Vitamin D) [20]	Improves calcium absorption, essential for bone mineralization and overall bone health.	Increases the expression of the vitamin D receptor in intestinal cells, improving calcium absorption.
Immune Modulation (IL-10, TGF-β) [13, 16, 15, 17]	Decreases osteoclast activity and promotes bone formation by increasing the production of anti-inflammatory cytokines.	Promotes the expansion of Treg cells that secrete IL-10 and TGF-β, modulating the immune response to protect against bone loss.
Strengthening of the Intestinal Barrier (SCFAs, Butyrate)[13, 15, 17]	Strengthens the intestinal barrier, reduces intestinal permeability, and protects against systemic inflammation that negatively affects bone health.	Produces SCFAs like butyrate, which improves intestinal barrier function, reducing endotoxin translocation and systemic inflammation.
Regulation of Osteoprotegerin (OPG) [17]	Inhibits osteoclast formation and reduces bone resorption, promoting bone formation.	Increases the expression of OPG, a decoy receptor that blocks the interaction of RANKL with RANK, thus decreasing osteoclastic activity.

6.3 Gut - brain - bone axis

The gut-brain-bone axis represents a complex network of communication between the gut, the central nervous system, and the skeletal system. This axis integrates biochemical and microbial signals from the gut that can influence brain function and bone health [38].

In this research, various metabolic pathways and their metabolites, as well as the bacteria that produce or interact with them, have been explored and related within the axis. Metabolites such as SCFAs, neurotransmitters like GABA, and hormones like osteocalcin play critical roles in modulating inflammatory processes, immune responses, and bone homeostasis.

This table provide a comprehensive resource aimed to improving the understanding of the axis and its implications with the human health. One particularly pathway highlighted in the table is the RANKL and TRAP5. This pathway is integral dedicated to osteoclastogenesis —the process by which bone-resorbing cells (osteoclasts) are formed. The inhibition of this pathway by butyrate, not only reduces bone loss but also promotes bone formation. This dual action makes it a particularly promising target for therapeutic interventions.

Moreover, the connection between serotonin signaling and bone remodeling is another area for exploration. Serotonin, commonly associated with mood regulation, also influences osteoblast and osteoclast activity, linking mental health and bone density in a way that is not yet fully understood. The cross-talk between serotonin production in the gut and its effects on the skeletal system could open new avenues for research, particularly in understanding how stress and mental health conditions contribute to bone diseases.

After extracting the results related to the gut-brain-bone axis from a total of 23 articles, it was decided to place this field of the research in the background. Given the methodology followed in this thesis, it was concluded that continuing with the development of this database based on 23 articles would not be immediately beneficial, and a systematic review exclusively dedicated to this axis could provide more precise understanding. Therefore, as a potential future improvement, conducting a systematic review specifically focused on the gut-brain-bone axis is proposed. This would involve applying the same comprehensive process used in this thesis for the gut-bone axis, but with a particular emphasis on the gut-brain-bone axis.

Table 6.3. Summary of metabolic pathways, effects, involved metabolites and participating microbiota in the gut-brain-bone axis.

Metabolic pathway	**Effect and relation with the gut-brain-bone axis**	**Participating microbiota**
RANKL and TRAP5 Pathways [11]	Inhibition of these pathways by butyrate reduces osteoclastogenesis, promotes bone formation, improves bone mineral density, and reduces bone loss.	*Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus paracasei*
Anti-Inflammatory Pathways [11, 37]	SCFAs (butyrate) reduce inflammatory cytokines (IL-6 and TNF-α), improving bone and brain health by reducing systemic inflammation.	*Lactobacillus reuteri*, *Bifidobacterium longum*, *Faecalibacterium prausnitzii*
Treg-Th17 Modulation [9, 37]	GABA and butyrate regulate the differentiation of Treg and Th17 cells, balancing the immune response, which is essential for maintaining bone and brain health.	*Lactobacillus rhamnosus* (JB-1)
IGF-1 Signaling Pathway [11]	SCFAs enhance IGF-1 signaling, promoting bone formation and growth by connecting gut and brain signaling, which improves bone density and strength.	*Bifidobacterium longum*, *Bacteroides spp.*
Serotonin Signaling [37]	Serotonin regulates osteoblast and osteoclast activity, influencing bone density and quality through central and peripheral signaling, ensuring balanced bone remodeling.	*Escherichia coli*
G-protein-coupled receptors (GPCR) - GPR41, GPR43, GPR109A [35]	SCFAs interact with GPCRs to regulate renin release and blood pressure, indirectly benefiting bone health through anti-inflammatory effects.	*Lactobacillus murinus*, *Bacteroides spp.*
Olfactory Receptor 78 (Olfr78) Involved metabolite: SCFAs (Propionate)	Propionate promotes vasodilation by altering Olfr78 and GPR41 activity, contributing to an acute hypotensive response and indirectly benefiting bone health.	*Bacteroides spp.*
GABA Production [35, 9]	GABA regulates brain function, reduces anxiety, and improves mood, indirectly benefiting bone health by reducing stress-related bone loss.	*Lactobacillus rhamnosus* (JB-1), *Streptococcus thermophilus*
Osteocalcin Production [11, 37, 9]	Osteocalcin, along with vitamin K and SCFAs, directly influences bone formation and homeostasis, preventing fractures and maintaining bone health.	*Lactobacillus reuteri*, *Bacteroides spp.*, *Faecalibacterium prausnitzii*
Neuromodulator Production [9]	Neurotransmitters like serotonin and dopamine regulate mood and behavior, impacting bone health by influencing the stress response and ensuring balanced bone remodeling.	*Escherichia coli*, *Lactobacillus spp.*
Dietary Fiber Fermentation [37, 35]	SCFAs produced during fiber fermentation regulate inflammation, improve intestinal permeability, and enhance calcium absorption, all critical for bone health.	*Faecalibacterium prausnitzii*, *Bacteroides spp.*, *Lactobacillus spp.*, *Clostridium spp.*
Corticotropin (CRH) Signaling [37, 11]	CRH plays a key role in regulating the stress response, with chronic stress negatively affecting both brain and bone health. SCFAs and other metabolites help mitigate these effects.	*Lactobacillus spp.*, *Bifidobacterium spp.*, *Firmicutes*, *Tenericutes*

6.4 Phylobone Project

The Phylobone project [2], developed in collaboration with the research group at the University of Turku, focuses on bone regeneration from an evolutionary and biological perspective. One of the main tools of this project is its database, which contains an extensive collection of proteins related to bone health. Given the focus of our study, it was decided to compare the Phylobone protein database with the articles collected in our research. The objective was to identify the most relevant proteins in recent studies on bone health and gut microbiota.

From this comparison, the following results were obtained, showing the frequency with which each protein appears in the reviewed literature. As can be seen, the most interesting proteins in recent years are Cathepsin K and Osteopontin, which we will discuss in detail below.

Table 6.4. Frequency of relevant proteins according to the Phylobone database, with corresponding PBID.

Protein	**PBID**	**Frequency**
Cathepsin K	PB0100	22
Osteopontin	PB0102	18
Fibroblast growth factor 23	PB0181	5
Plasminogen	PB0238	5
Matrix GLA protein	PB0004	4
Matrix metalloproteinase-9	PB0107	4
Apolipoprotein E	PB0179	3
Glyceraldehyde-3-phosphate dehydrogenase	PB0011	2

The dominance of Cathepsin K and Osteopontin in the Table 6.4 suggests that these proteins play important roles in bone remodeling and resorption processes. The lower frequency of other proteins like Fibroblast growth factor 23 and Matrix GLA protein may indicate emerging areas of interest for further investigations.

6.4.1 Osteopontin

Osteopontin is a key protein in bone remodeling and immune regulation. Its main action in the body is to mediate the adhesion of osteoclasts to the bone matrix, facilitating bone resorption and remodeling [14]. Additionally, Osteopontin plays a significant role in modulating immune responses, particularly through metabolic pathways such as NF-κB, RANK/RANKL, MAPK, Runx2, and TAZ/IHH [40, 14].

This protein is influenced by several substrates, including butyric acid and gangliosides present in milk [41]. Although specific strains are not mentioned, some probiotics involved in its activity are *Faecalibacterium prausnitzii*, *Lactobacillus reuteri*, *Bifidobacteria*, and *Dialister* [41]. The Osteopontin promotes bone formation, prevents bone loss, and contributes to the maintenance of intestinal integrity [41].

Table 6.5. Summary of metabolic pathways related to Osteopontin in bone health

Metabolic Pathway	**Effect in Bone Health and Metabolites Involved**	**Related Microbiota**
NF-κB Signaling	Promotes osteoclastogenesis by activating osteoclasts, leading to bone resorption. Inflammatory cytokines like TNF-α enhance the expression of Osteopontin, further promoting resorption [40].	*Lactobacillus reuteri*, *Bifidobacteria* [40]
RANK/RANKL Signaling	Facilitates osteoclast differentiation and activation, leading to bone resorption. Osteopontin binds to integrin receptors on osteoclasts, enhancing their adhesion to bone matrix [14, 40].	*Faecalibacterium prausnitzii*, *Lactobacillus reuteri* [14, 40]
MAPK Pathway	Regulates osteoclast activity and expression of Osteopontin, contributing to bone resorption. It also impacts osteoblast activity through the production of growth factors like TGF-β [14].	*Faecalibacterium prausnitzii*, *Dialister* [14]
TGF-β/BMP Pathway	Modulates osteoblast differentiation and activity, promoting bone formation. Osteopontin acts as an anti-apoptotic factor for osteoblasts, favoring continuous bone formation [41].	*Faecalibacterium prausnitzii*, *Bifidobacteria* [41]
WNT Signaling	Enhances osteoblast differentiation and bone formation through the regulation of gene expression related to bone development. Osteopontin is involved in maintaining osteoblast survival [14, 41].	*Lactobacillus reuteri*, *Bifidobacteria* [14, 41]
TAZ/IHH Pathway	Contributes to the regulation of osteogenesis and the maintenance of bone matrix, supporting the role of Osteopontin in bone formation [14].	*Faecalibacterium prausnitzii* [14]

6.4.2 Cathepsin K

Cathepsin K is a protease that participates in the degradation of Type I collagen during bone resorption. Its activity increases under conditions of obesity due to elevated systemic inflammation, leading to enhanced differentiation of osteoclasts and consequently greater bone resorption [7]. This process is driven by pro-inflammatory cytokines such as TNF-α and IL-1β, which upregulate Cathepsin K expression, resulting in a reduction in BMD [7].

Additionally, microbe-associated molecular patterns (MAMPs) also modulate Cathepsin K activity. LPS from gram-negative bacteria activates the TLR4 receptor, increasing the production of inflammatory cytokines like IL-1β and TNF-α, which in turn stimulates osteoclast activity and bone resorption [42]. On the other hand, SCFAs, products of bacterial metabolism in the gut, can inhibit osteoclast differentiation at low doses, promoting osteogenic activity and suggesting a potential protective effect on bone health [42].

Table 6.6. Metabolic pathways of Cathepsin K and their impact on bone health

Metabolic pathway	**Metabolites and effect on bone health**	**Effect on bone health**
Inflammation induced by obesity [7]	TNF-α, IL-1β: These cytokines increase Cathepsin K activity, enhancing collagen degradation and osteoclast differentiation, leading to increased bone resorption and decreased BMD.	Promotes osteoclastogenesis, leading to increased bone resorption and decreased BMD.
Signaling by LPS [42]	IL-1β, IL-6, TNF-α: LPS induces these cytokines, which stimulate Cathepsin K activity, boosting osteoclast activity and accelerating bone matrix breakdown.	Promotes osteoclastogenesis, leading to increased bone resorption.
MAMPs [42]	TRAP, MMP-9, Runx2, osterix: SCFAs, products of bacterial metabolism, inhibit osteoclast differentiation at low doses, reducing Cathepsin K activity, thus supporting bone formation and promoting osteogenic activity.	Promotes osteogenic activity, providing a protective effect on bone health.

7 Discussion

The analysis of the results obtained in this research has allowed to establish a solid relationship between the gut microbiota, the metabolites produced, and their impact on bone health, as well as the interactions with the brain through the gut-brain-bone axis. Throughout this thesis, the proposed objectives have been effectively achieved. First, the role of metabolites and microbiota in the gut-bone axis was evaluated, identifying probiotics and metabolites that influence bone health. Second, the metabolic pathways involved in the gut-brain-bone axis were investigated, highlighting the interrelationship and complexity of these systems. Third, a comparison was made between the Phylobone database and the data obtained in this review, identifying frequent proteins and probiotics and enriching the database with new findings. Finally, it was verified that the technology and automation processes used accelerated the research, allowing for the identification of potential therapies or interventions in the biomedical field.

Throughout this thesis, a better understanding of the interactions between the gut microbiota, the metabolites it produces and their infleunce on bone health has been achieved. It also explores the gut-brain-bone axis, an area of research still in development. The identification of possible connections between the gut microbiota and the central nervous system, and its impact on bone regulation, opens new paths for research and clinical applications, especially in neurodegenerative disorders associated with bone density loss.

This study has enabled the collection and organization of a substantial amount of data into tables, which not only summarize the findings and facilitate their visualization but also highlight key aspects of interest, such as the probiotic LGG (Table 6.2) and the metabolites Osteopontin (Table 6.5) and Cathepsin K (Table 6.6). This approach contributes to more robust conclusions and the identification of potential therapeutic targets to enhance bone health.

However, it is important to note some limitations of this study. The quality of the results has progressively improved throughout the research development, resulting in greater content in the sections on probiotics or Phylobone compared to the bacteria table (Fig 6.1). This reflects the advancement of the system used but also indicates that the initial findings may not be as exhaustive as those obtained in later phases. This aspect should be noticed when interpreting the results and considering the replication of the study in future work. Additionally, some troubles were found in accessing some full-text articles due to institutional restrictions, which led to the exclusion of potentially relevant studies.

To comprehensively visualize the development and connections established in this research, a triple network graph has been used (Fig 7.1). This graphic illustrates the interactions between bacteria, metabolites, and the proteins present in the Phylobone database, providing an overview of the complex network of relationships that influence bone health. The triple network graph shows the interactions between native bacteria, metabolites, and bone-related proteins from the Phylobone database. Blue nodes represent bacteria, green nodes proteins, and red nodes metabolites. The lines connecting the nodes indicate interactions, the nodes closer to the center have more connections, highlighting their central role in the network. Nodes farther from the center indicates a more peripheral role in the network.

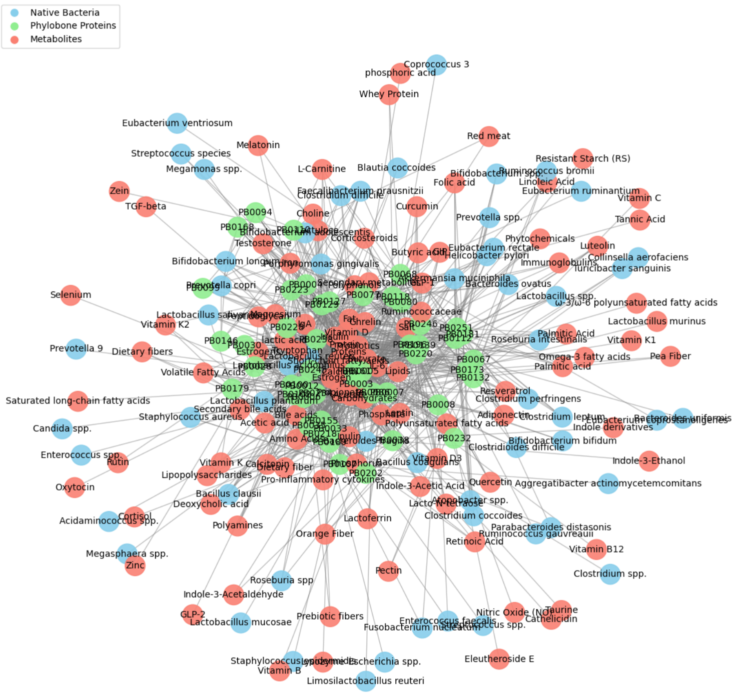

Figure 7.1. Phylobone network illustrating the relationships between gut microbiota (blue nodes), metabolites (red nodes), and ECM proteins(green nodes).

This approach not only synthesizes the results obtained but also offers a starting point for future research that seeks to study these relationships and develop new therapeutic interventions. The ability to visualize and understand these interactions is crucial for advancing the development of innovative and effective therapies in the field of bone health.

Conclusion

The purpose of this research was to explore and analyze the relationship between the gut microbiome and bone health, with the aim of identifying possible metabolic pathways and probiotics that could develop more effective therapies for bone diseases, such as osteoporosis. Through an automated systematic review, the study aimed to map the gut-bone axis and evaluate the utility of automation in scientific research.

The most significant results of this study include the identification of microbiota and metabolites involved in bone health, compiled into a database that provides an overview of the gut-bone axis. A key finding was the role of LGG, whose positive impact on bone health was documented, revealing the metabolic pathways it participates and demonstrating its therapeutic potential. These results confirm the hypothesis proposed at the beginning of the research: the gut microbiome plays a crucial role in bone health and has the potential to be modulated to develop more effective therapies.

One of the challenges encountered during the research was the complexity of automating the systematic review process. Despite the progressive improvements in the algorithms used, the initial results did not provide as much information as those obtained in later stages. This highlights the need to continue refining the codes and tools used, ensuring that automation is as effective as possible. This method of automation has the potential to be applied to other systematic reviews, which could revolutionize how scientific research is approached, making it more efficient and less prone to human error. Additionally, sometimes it was not possible to obtain the full-text PDFs of the best articles due to institutional restrictions or private publications, which led to the exclusion of some potentially relevant studies from their analysis.Furthermore, this review needed the inclusion of an extensive number of references, surpassing the maximum limit.

The objectives established at the start of the thesis have been achieved. Not only a detailed mapping of the axis has been accomplished, but the interactions between microbiota, metabolites, and bone health were also confirmed, integrating these findings also in another context with the collaboration of Phylobone. This allowed the identification of key proteins in the bone extracellular matrix and their relationships with the metabolites and microbiota of the axis, facilitating the connection of the obtained results.

Looking for next steps, it would be relevant to explore the identified probiotics and metabolic pathways to develop new therapeutic strategies. Additionally, it would be beneficial to conduct this systematic review again with improved algorithms to obtain even more precise and comprehensive results. Furthermore, a dedicated review of the gut-brain-bone axis could also be conducted, as this area was only addressed collaterally in the review, leaving room for more exploration.

Autoevaluación

Realizar esta tesis ha sido un proceso de aprendizaje y crecimiento, tanto a nivel personal como profesional. Desde el primer momento, el proyecto me llamó la atención; la posibilidad de descubrir nuevas terapias contra la osteoporosis resultaba prometedora. Después de la reunión con Pere y de examinar el material inicial para comenzar la revisión sistemática, el modelo PRISMA captó mi interés, y me di cuenta de que muchos de sus pasos se podían automatizar, lo que me motivó a aplicar mis conocimientos en ingeniería informática para optimizar el proceso.

La automatización del proceso fue un desafío, pero ver cómo el sistema iba mejorando progresivamente me motivó a seguir adelante. Inicialmente, logré obtener una columna de palabras clave específicas a partir del título y el abstract de los artículos, lo que permitió definir claramente cada estudio. Posteriormente, apliqué criterios específicos para determinar qué artículos tenían mayor similitud con cada categoría de interés y, finalmente, implementé un filtrado iterativo booleano que clasificó los estudios en función de su relevancia, identificando aquellos que podían aportar más información valiosa. Con cada paso, los algoritmos se volvieron más precisos y capaces de extraer más información, lo que hizo que los resultados obtenidos en etapas posteriores fueran mejores en comparación con los iniciales.

Considero que el trabajo es muy completo. No solo se logró mapear las interacciones del eje intestino-hueso, recopilando toda la microbiota y metabolitos involucrados en una base de datos, sino que también se identificaron rutas metabólicas específicas y probióticos que interactúan con ellas. Un ejemplo es el Lactobacillus rhamnosus GG, cuyo impacto en la salud ósea ha sido documentado en la tesis, exponiendo las rutas metabólicas en las que participa y mostrando su potencial en la mejora de la salud ósea. Además, la colaboración con el proyecto Phylobone permitió integrar estos hallazgos en un contexto más amplio, utilizando su base de datos para identificar proteínas clave en la matriz extracelular ósea y cómo se relacionan con metabolitos y microbiota del eje.

Estoy satisfecho con los resultados logrados. La tesis no solo cumple con los objetivos establecidos, sino que también demuestra cómo la intersección entre la biotecnología y la informática puede generar soluciones innovadoras y efectivas en la investigación científica. Haber fusionado estas dos áreas para desarrollar este proyecto me ha mostrado cómo ambas disciplinas pueden integrarse. Este trabajo marca el punto de convergencia entre dos carreras que prácticamente se estudian por separado, dándole sentido a todo el conocimiento adquirido para crear un proyecto sólido en el campo de la investigación científica a través de herramientas de ingeniería.

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A Appendices

A.1 BIB Format

Below is an example of a BIB format entry exported via Sciwheel, which organizes reference data into fields, making it compatible with Python scripts and other tools.

```
@article{fontcubertarigo_2023,
        title = {Phylobone: a comprehensive database of bone
           extracellular matrix proteins in human and model
           organisms.},
        author = {Fontcuberta-Rigo, Margalida and Nakamura, Miho
            and Puigb\'o, Pere},
        pages = {44},
        url = {http://dx.doi.org/10.1038/s41413-023-00281-w},
        year = {2023},
        month = {aug},
        day = {15},
        urldate = {2024-08-31},
        journal = {Bone research},
        volume = {11},
        number = {1},
        doi = {10.1038/s41413-023-00281-w},
        pmid = {37580331},
        pmcid = {PMC10425349},
        sciwheel-projects = {New Gut-Bone Papers},
        abstract = {The bone extracellular matrix (ECM) contains
            minerals deposited on highly crosslinked collagen
           fibrils and hundreds of non-collagenous proteins.
           Some of these proteins are key to the regulation of
           bone formation and regeneration via signaling
           pathways, and play important regulatory and
           structural roles. However, the complete list of bone
           extracellular matrix proteins, their roles, and the
           extent of individual and cross-species variations
           have not been fully captured in both humans and model
            organisms. Here, we introduce the most comprehensive
            resource of bone extracellular matrix (ECM) proteins
            that can be used in research fields such as bone
           regeneration, osteoporosis, and mechanobiology. The
           Phylobone database (available at https://phylobone.
           com) includes 255 proteins potentially expressed in
           the bone extracellular matrix (ECM) of humans and 30
           species of vertebrates...}}
```

A.2 Phylobone Database and Website

Phylobone Database on GitHub - https://github.com/tomascan/Phylobone-Database
Download CSV Tables - https://phylobone.com/PUBLICATIONS/canton_2024.zip

- A comprehensive database of all relevant data.
- All tables used in the analysis.
- A website with the complete bibliography referenced throughout this thesis.
- CSV files of all tables used in the analysis.

A.3 Search Queries

Dataset 1: Native bacteria related to the gut-bone axis

This table focuses on identifying bacterias in the gut that are related to bone health. This process reduced the list to 304 articles.

```
("Gut" AND "Bone") AND ("Bacteria" OR "Microbiota"
    OR "Strain" OR "Osteogenesis" OR "
   Osteoclastogenesis" OR "Lactobacillus" OR "
   Bifidobacterium" OR "Faecalibacterium" OR "
   Bacteroides" OR "Streptococcus" OR "Clostridium
   " OR "Eubacterium" OR "Akkermansia" OR "
   Propionibacterium" OR "Bone Health")
```

Query 1. Search string for gut microbiota and bone health.

Dataset 2: Probiotic strains and bone health

This table seeks information on external probiotic strains and their impact on bone health. This filtering resulted in 86 articles.

```
("Probiotics" AND "Gut" AND "Bone") AND ("Strain"
   OR "Osteoblast" OR "Osteoclast" OR "Bacillus"
   OR "Lactobacillus" OR "Bifidobacterium" OR "
   Saccharomyces" OR "Probiotic" OR "Osteogenesis
   ")
```

Query 2. Search string for probiotics and bone health.

Dataset 3: Metabolites and metabolic pathways

This table includes articles detailing how proteins and other molecules affect bone regeneration and destruction, reducing the total to 247 selected articles.

```
("Gut" AND "Bone") AND ("Proteins" OR "Peptide" OR
    "Molecule" OR "Metabolite" OR "Cytokine" OR "
   Hormone" OR "Signaling" OR "Pathway" OR "Biotic
   " OR "Osteogenesis" OR "Bone Formation" OR "
   Bone Resorption" OR "Bone Health")
```

Query 3. Search string for metabolites and bone health.

Dataset 4: Gut-brain-bone interactions

For the final table, studies that connect the gut microbiota, brain, and skeleton were considered. This filtering led to the identification of 35 relevant articles.

```
("Gut" AND "Bone" AND "Brain") AND ("Protein" OR "
   Hormone" OR "Matrix" OR "Extracellular" OR "
   Health" OR "Bacteria" OR "Microbiota" OR "Neuro
   " OR "Path")
```

Query 4. Search string for gut-brain-bone interactions.