

Review Article

# A Comparative Systems Analysis of Non-Enzymatic Metabolism in Human and Bacterial Networks

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## Abstract

The metabolism is divided into two types: enzymatic and non-enzymatic. The non-enzymatic metabolism is, a group of biochemical reactions that occur spontaneously or are catalyzed by small molecules or light and, do not rely on enzymes for catalysis. This type of metabolism contributes to the formation of certain reactive metabolites, modifies proteins, and affects cell stability through chemical pathways independent of enzyme catalysis. Databases used in metabolic research can be classified into four main types: IBM SPSS version 25 for statistics, Recon 2.2, iML1515 as a data base, KEGG and MetaCyc to understand metabolic pathways, and Colab Research for drow charts. The metabolic networks in humans and *E. coli* are dominated by enzymatic reactions; the relative proportion of non-enzymatic reactions is higher in *E. coli* ( $\approx 1-5\%$ ) compared to humans ( $\approx 0.1-1\%$ ), reflecting the simplicity of cellular organization and the high influence of spontaneous chemistry in primitive systems. This study provides a preliminary quantitative estimate of the contribution of non-enzymatic interactions to reconstructed metabolic networks in humans and *Escherichia coli*. The results show that this contribution remains limited but, tends to increase significantly in evolutionarily simpler systems. The study proposes a methodological framework that can serve as a basis for future research exploring the impact of these interactions on cellular metabolic dynamics. This study aims to conduct a comparative analysis of non-enzymatic interactions in human and bacterial metabolic networks, assessing their distribution within different metabolic systems, based on global metabolic databases such as Recon and KEGG.



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## 1. INTRODUCTION

Metabolism is the set of chemical reactions necessary for sustaining life within living organisms. It includes essential functions such as energy conversion, macromolecule synthesis, and waste elimination. These reactions are regulated within metabolic pathways, each of which specialized enzymes act upon, functioning as highly efficient catalysts. This allows for the acceleration and precise adjustment of required reactions in response to cellular needs and changes in the surrounding environment [1, 2]. The resilience of metabolic systems and the crucial role of enzyme catalysis in the survival of organisms are illustrated by the evolution of these enzymes to cope with extreme conditions, such as high or low temperatures [3, 4]. Traditional metabolism models have focused on enzyme-catalyzed reactions, but recent studies have shown that spontaneous, enzyme-independent reactions, termed "non-enzyme metabolism," are more common than previously assumed [5]. Metabolism from this type that do not require specialized enzymes was spontaneous biochemical transformations, or those catalyzed by small molecules or light. These reactions have been found to influence protein modification, the formation of reactive metabolites, and the regulation of cellular responses to oxidative stress, and even interact with enzymatic pathways in ways that reflect unexpected regulatory dynamics [5, 6].

Recently, studies on oxidation-reduction regulation have shown that many of these non-enzymatic reactions, especially those involving reactive oxygen species and sulfur species, play important roles in maintaining cellular homeostasis and in the onset of chronic diseases when they are dysregulated [7]. From an evolutionary perspective, the "metabolic fingerprint" hypothesis posits that some of these non-enzymatic reactions are remnants of primitive metabolic systems that prevailed before the emergence of highly specialized proteolytic enzymes. Chemical and biological data support this hypothesis by demonstrating that central metabolic pathways, including glycolysis and the pentose phosphate pathway, can be spontaneously triggered under conditions that could have existed in the early stages of life. Wherefore, the study of non-enzymatic metabolism in modern organisms is not limited to understanding side reactions; it may also provide a window into how metabolic networks arose and evolved [8].



Although important, the contribution and distribution of non-enzymatic interactions in the reconstructed metabolic networks of modern organisms are still incompletely quantified. The importance of this question is growing with the increasing body of evidence that small molecules such as hydrogen sulfide and reactive sulfur species are involved in cellular metabolic regulation not only as by-products but also as active players. There is a need for systematic tools to monitor these interactions and to separate them from the background of complex enzymatic activity [3]. This study aims to conduct a comparative quantitative assessment of the prevalence of non-enzymatic versus enzymatic interactions within reconstructed metabolic networks in humans and *Escherichia coli* using, genome-wide metabolic models, metabolic pathway databases, and computational statistical analysis. The study seeks to explore the distribution of these interactions across different metabolic subsystems and test the hypothesis of their relative prevalence in evolutionarily simpler networks, thereby contributing to a better and more comprehensive understanding of non-enzymatic metabolic processes within complex cellular environments.

## 2. METHOD

Databases used in metabolic research can be classified into four main types:

### (a) Genome-scale metabolic models.

The study employed multimetabolic models, such as Recon 2.2 and iML1515, representing metabolic interactions at the genome level and link genetic information to cellular physiological functions. It was used to analyze the distribution of non-enzymatic interactions within human metabolic networks [9].

### (b) Metabolic Pathway Database.

Specialized metabolic databases such as KEGG and MetaCyc were used, providing structured information on metabolic pathways, interactions, enzymes, and associated metabolites. These databases were used to compare and classify metabolic interactions and identify non-enzymatic interactions within different pathways [10].

### (c) Statistical analysis and data representation tools

The statistics were simple during this study and were not that complex. Despite this, IBM SPSS Statistics software was used to analyze the metadata and statistical comparisons, while Google Colab was used to create graphs and visually represent the data.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

Recon 2.2 from the genome-wide metabolic network model (Figure.1) [11]. The Recon 2.2 model contains 7,785 interactions collected manually and computationally from major databases, such as KEGG and MetaCyc, and 1,675 associated genes. For comparison with bacteria, the newer and more comprehensive E-coli iML1515 model [12], which includes 2,719 interactions and 1,515 genes, was used. This model is the most accurate reference model for this organism to date.

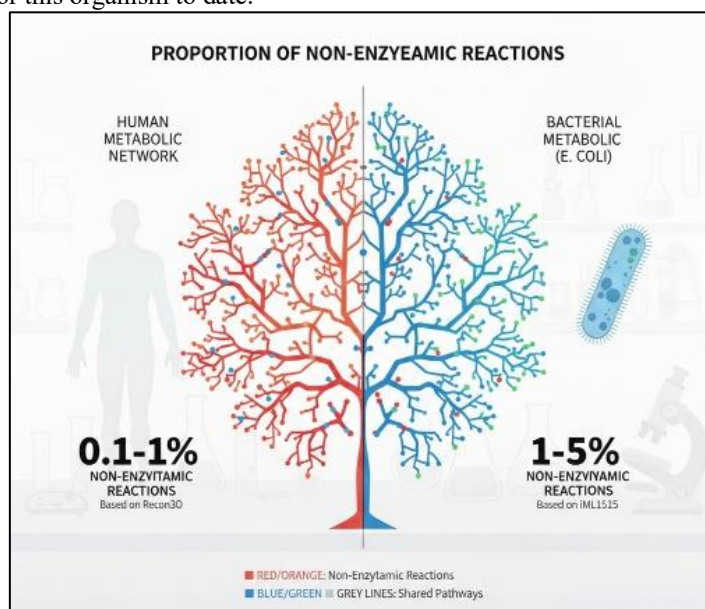


Figure (1): A quantitative map showing the proportion of non-enzymatic reactions that can occur in the human/bacterial metabolic network.

Although this study uses large metabolic reconstructions, such as Recon 2.2 and iML1515, non-enzymatic reactions are still feasible based on thermodynamic principles. However, they are not as efficient as enzyme-catalyzed reactions, as enzymes can lower activation energy and steer reactions down biological pathways [12, 13]. The (Table1) below shows the number and the percentages of reactions studied in the database and compared between humans and bacteria, including enzymatic and non-enzymatic reactions extracted from the models.

Table (1): Antibiotic disks and inhibition zones (CLSI, 2024)

| Metric                          | Human          | E. coli        | Difference (E. coli – Human) |
|---------------------------------|----------------|----------------|------------------------------|
| Total Reactions                 | 7,000          | 2,719          | –4,281                       |
| Enzymatic Percentage            | 99.0–99.9      | 95.0–99.0      | –0.9 to –4.0                 |
| Non-enzymatic Percentage        | 0.1–1.0        | 1.0–5.0        | +0.9 to +4.9                 |
| Non-enzymatic Reactions (count) | 7–70           | 27–136         | +20 to +66                   |
| Enzymatic Reactions (count)     | 6,930–6,993    | 2,583–2,692    | —                            |
| Statistical CI                  | Not applicable | Not applicable | —                            |
| Literature-supported Range      | 0.1–1.0        | 1.0–5.0        | —                            |
| Reference                       | [11]           | [12]           | —                            |

As for the Statistical CI (Confidence Interval) shown in (Table 1), it is a mathematically calculated numerical range used to indicate that the most common CI is 95%. Therefore, it could not be measured here within the table, as CI is not estimated from a "literature range" or "scientific guess." CI requires actual data. The number of metabolic reactions was taken from the iML1515 and Recon 2.2 databases, and these exclude all reactions, but only those recorded in these databases. (Figure.2)

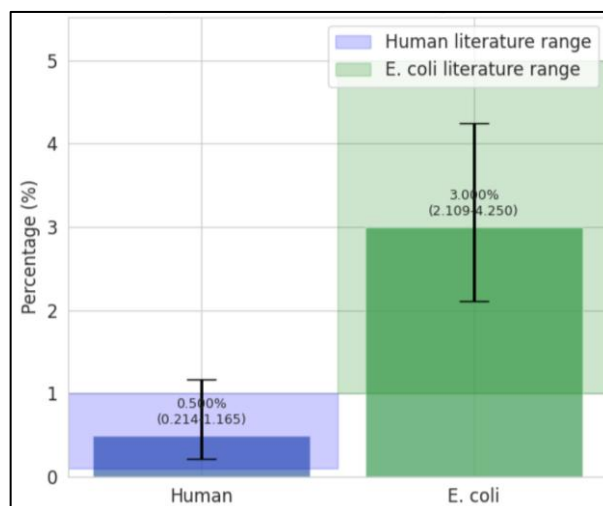


Figure (2): Illustrates the difference (E. coli – Human) in non-enzymatic reactions that can occur in the human/bacteria.

In (Figure 3), the data show that some pathways, such as the urea cycle and oxidative phosphorylation in humans, correspond to purine and pyrimidine metabolism and fatty acid oxidation in bacteria, within different susceptibility ranges.

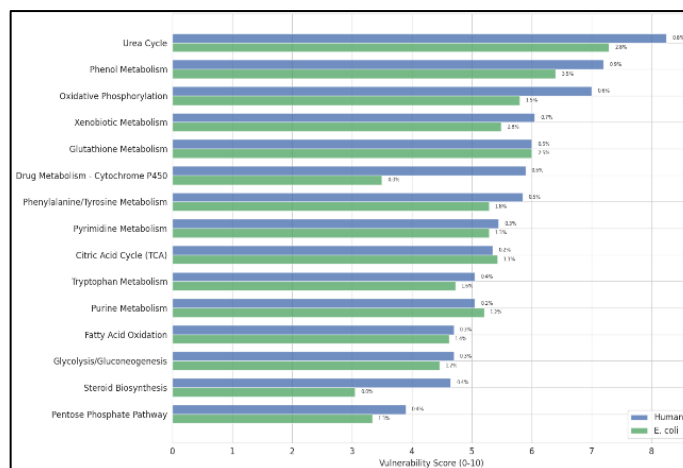


Figure (3): Pathway Vulnerability Comparison: Human vs E. coli (non-enzymatic percentages).

Figure (4) shows the relative values representing the pathways associated with non-enzymatic reactions, where the percentage of phenol metabolism and foreign compound metabolism in humans was 1.2% and 1.0% compared to 3.4% and 2.6% in bacteria, respectively.

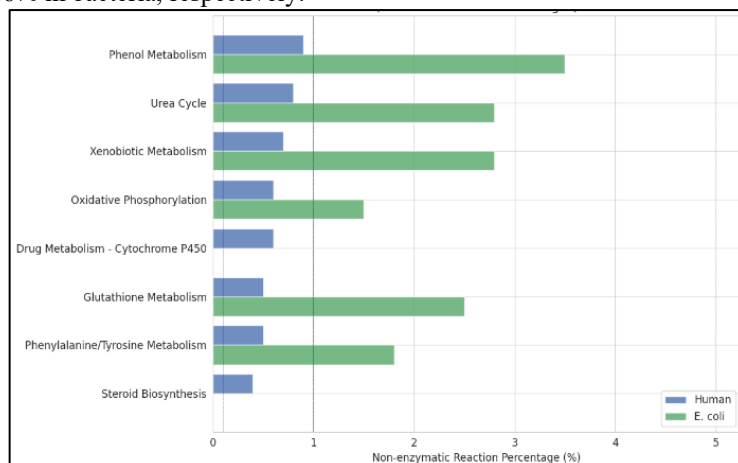


Figure (4): Top Pathways by Non-Enzymatic Reactions

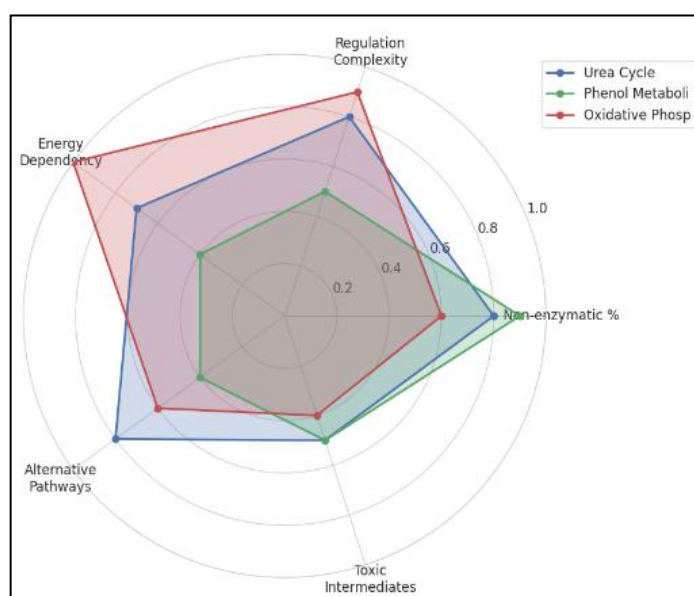
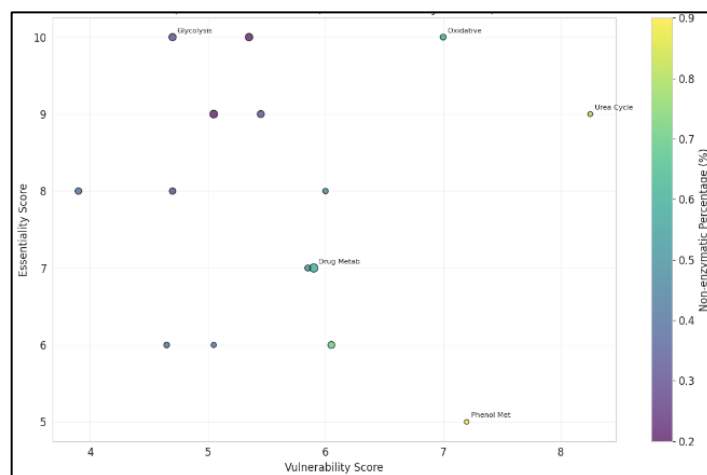


Figure (5): Vulnerability Factors for the Top 3 Vulnerable Pathways.

Figure (5) shows that the urea cycle is the most susceptible to disruption due to its high energy dependence, the lack of alternative pathways, and the semi-automatic nature of the reactions. In contrast, oxidative phosphorylation carries a high toxicity risk due to the production of free radicals and its central role in energy production.



\*Size Reaction count, Color = Non-enzymatic %

Figure (6): Five major metabolic pathways were assessed based on three quantitative criteria: susceptibility, importance/necessity, and the percentage of non-enzymatic interactions.



Differences in the representation of pathways associated with non-enzymatic reactions suggest that they are more likely influenced by the construction characteristics of the metabolic models used and the level of documentation within databases than by direct reflection of intracellular physiological metabolic activity, as shown in (Figure 3) [19]. The high representation reflects the proportion of certain pathways involved in the metabolism of foreign compounds in bacteria, indicating a greater ability to adapt to environments containing foreign compounds; however, this interpretation remains within the scope of model analysis and is not a direct or definitive experimental conclusion [20]. This is because pathways that are more functionally important tend to have regulatory and compensatory mechanisms that decrease their sensitivity to perturbation, while specialized pathways are more sensitive to environmental or chemical changes.

These observations are consistent with the general concept in metabolic network science that central pathways possess higher regulatory stability than peripheral or specialized pathways. The results also demonstrate that pathways with high functional value and low sensitivity, such as purine and pyrimidine metabolism, are essential components of biological system stability, while pathways with high sensitivity may represent potential targets in pharmaceutical applications, as shown in (Figure 7) [21].

The urea cycle is a highly sensitive pathway due to its high energy dependence and the accumulation of nitrogenous toxins. Xenodrug/cytochrome P450 metabolism pathways are also affected by changes in enzyme and cofactor levels, while glutathione metabolism is associated with oxidative stress [22].

Oxidative phosphorylation is affected by mitochondrial dysfunction and hypoxia, while central regulatory mechanisms govern the citric acid cycle (TCA) with the potential for metabolic rerouting. Glycolysis exhibits regulatory flexibility but is sensitive to changes in the metabolic environment [23].

Purine and pyrimidine metabolism is essential for DNA synthesis and is finely regulated, while the pentose phosphate pathway provides NADPH and ribose and works along with other metabolic pathways. Folic acid synthesis is also a key pathway in the synthesis of nucleotides and amino acids [24].

#### 4. CONCLUSION

In this article, non-enzymatic reactions are lumped together, including passive diffusion through membranes, spontaneous chemical reactions, some equilibrium chemical reactions, and intrinsic redox. The results yielded a table and several figures indicating that data from metabolic network models show differences in the representation of this type of interaction among the studied organisms. Bacteria appear to exhibit a relatively higher representation of non-enzymatic interactions compared to humans in the databases used. Confirm that these values were adopted based on the database used; however, these values are not necessarily final. This observation reflects a difference in the structure of metabolic networks and the level of documentation within the models rather than being a direct indicator of actual intracellular metabolic activity. The limitations of this study rely on data extracted from established metabolic modeling frameworks, which do not cover all possible biochemical reactions within living systems. Furthermore, the classification of non-enzymatic reactions depends on definitional criteria within these models rather than direct experimental measurements. In addition, the study lacks kinetic or intracellular experimental data, limiting the generalizability of the findings to the entire physiological level.

#### RECOMMENDATIONS

- Develop better methods for detecting non-enzymatic reactions in vivo.
- Investigate the potential regulatory role of these reactions.
- Explore applications in biotechnology (reactions without expensive catalysts).
- Integrate these reactions into genome-wide metabolic models.

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#### BIOGRAPHIES OF AUTHORS

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