

In-Silico Assessment of Erythritol Interactions with Microbial Enzymes Involved in Short-Chain Fatty Acid Metabolism

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Abstract:

Erythritol is a low-calorie polyol often used as a sugar substitute. While most of the ingested erythritol is absorbed in the small intestine and excreted unchanged [1-3], recent research has demonstrated that erythritol has different effects on microbial metabolites, such as short-chain fatty acids (SCFAs). The chemical basis of these observations remains unknown. In the present work, the expected interactions of erythritol with certain microbial enzymes involved in acetate metabolism, butyrate synthesis and carbohydrate utilization were studied. Molecular docking was carried out against acetate kinase (PDB: 4H0O), butyrate kinase (PDB: 1SAZ), phosphotransbutyrylase (PDB: 7VG9), and fructose-6-phosphate phosphoketolase (PDB: 3AHD) using SwissDock 2024 with the AutoDock Vina scoring tool. Erythritol was chosen as the docking ligand and the protein structures were retrieved from the Protein Data Bank. For each protein-ligand system, twenty poses were generated, and the best ranking pose was chosen based on the projected docking score. Fructose-6-phosphate phosphoketolase had the highest projected score with a docking score of -4.778 kcal/mol. The scores of acetate kinase, butyrate kinase and phosphotransbutyrylase were -4.175, -3.783 and -3.716 kcal/mol respectively. Under the used technique the relative ranking was fructose-6-phosphate phosphoketolase > acetate kinase > butyrate kinase > phosphotransbutyrylase. All the four selected microbial enzyme models were expected to be compatible with erythritol. Fructose-6-phosphate phosphoketolase received the highest docking score. These data furnish a first structural hypothesis on possible interactions between erythritol and enzymes. Nevertheless, binding affinity, enzymatic inhibition and physiological effects on the gut microbiota cannot be identified by docking scores alone [6-8]. Validation involves microbiological research and experimental enzyme testing.

Keywords: erythritol; molecular docking; gut microbiota; short-chain fatty acids; acetate kinase; butyrate kinase; phosphotransbutyrylase; phosphoketolase.

1. Introduction

The human gut microbiota plays a major role in the metabolism of indigestible food substances, producing bioactive metabolites that will modulate the host's physiology. Of these metabolites, short-chain fatty acids (SCFAs) - mainly acetate, propionate and butyrate - are formed from the microbial fermentation of carbohydrates and proteins and function as energy substrates, signaling molecules and modulators of immunological and metabolic function. Acetate and butyrate are especially important for energy metabolism of colonic epithelial cells, maintenance of the intestinal barrier integrity and modulation of inflammation. [9–11].

The composition and metabolic output of the gut microbiome are largely determined by dietary trends. The increasing use of low- and no-calorie sweeteners (LNCS) instead of sugar has sparked questions about their impact on microbial populations and SCFA production. Erythritol, a four-carbon sugar alcohol, has gained popularity because of its near nil caloric value, little effect on blood glucose and insulin, and good sensory qualities [10, 19]. In humans, about 90% or more of the erythritol ingested is swiftly absorbed by passive diffusion in the small intestine and eliminated unchanged in the urine, with a minor fraction reaching the colon. [2, 19, 25].

However, despite this high absorption efficiency, a number of research have investigated whether the unabsorbed fraction of erythritol can interact with gut bacteria or impact SCFA profiles. In vitro investigations with human fecal microbiota showed no detectable fermentation of erythritol and no change in SCFA synthesis. However, other studies suggest that erythritol may boost the generation of butyrate and valeric acid under particular conditions without a significant change in overall microbial community structure. These seemingly contradictory data emphasize the need for a more comprehensive mechanistic understanding of how erythritol could interact with microbial metabolic pathways.

Structure-based molecular docking is a computational method for developing mechanistic hypotheses, by predicting the optimal orientation and interaction energy of a small molecule in the binding site of a target protein. Docking is a commonly utilized method in early stage drug development and in the study of enzyme-ligand interactions for the ranking of compounds for experimental testing. Programs like AutoDock Vina and web-based tools like SwissDock are based on empirical scoring functions that simulate van der Waals contacts, hydrogen bonding, hydrophobic effects, and conformational entropy penalties to predict the likelihood of favorable binding [5, 17, 23, 39].

While molecular docking cannot supplant empirical binding or activity measurements, it serves as a valuable computational tool for identifying plausible enzyme-ligand complexes and elucidating residues that may contribute to molecular recognition mechanisms. This approach proves particularly advantageous when examining non-nutritive sweeteners and polyols, which may engage with microbial enzymes through interaction patterns that remain obscured by phenotypic assays in isolation. Previous investigations have employed docking methodologies to elucidate erythritol interactions with carbohydrate-active enzymes, including α -glucosidase and α -amylase, thereby corroborating experimental findings regarding enzyme inhibition and postprandial glucose regulation [35].

Within the framework of short-chain fatty acid (SCFA) metabolism, several critical microbial enzymes warrant consideration. Acetate kinase (Ack) catalyzes the terminal phase of acetate generation from acetyl phosphate, while butyrate kinase (Buk) and phosphotransbutyrylase (Ptb) function synergistically to facilitate one biosynthetic pathway for butyrate synthesis originating

from butyryl-CoA. Additionally, phosphoketolases constitute essential components in carbohydrate metabolic pathways, particularly the phosphoketolase pathway identified in *Bifidobacterium* and related microbial taxa [3, 9, 40, 41]. The structural characterization of these enzymatic proteins has been documented in the Protein Data Bank (PDB), thereby facilitating structure-informed analyses of prospective substrate and ligand binding interactions. [30–32].

The current investigation utilized SwissDock 2024 in conjunction with the AutoDock Vina scoring algorithm to assess the predicted binding interactions between erythritol and four microbial enzymes implicated in acetate metabolism, butyrate biosynthesis, and carbohydrate utilization: acetate kinase (PDB: 4H0O), butyrate kinase (PDB: 1SAZ), phosphotransbutyrylase (PDB: 7VG9), and fructose-6-phosphate phosphoketolase (PDB: 3AHD) [4, 5, 40, 41]. SwissDock was selected due to its provision of an automated, reproducible web-based platform that integrates protein and ligand preparation with AutoDock Vina docking, and its demonstrated utility in numerous peer-reviewed investigations for analogous small-molecule docking applications [4, 5, 16]. The principal aim was to ascertain whether erythritol demonstrates favorable predicted docking scores against these targets and to establish a comparative ranking of interaction affinities across the four enzymes.

This investigation does not purport to establish that erythritol binds to or inhibits these enzymes *in vivo*, nor does it contend that erythritol directly regulates SCFA production in the human gastrointestinal tract. Rather, it furnishes a computational, hypothesis-generating examination that may inform subsequent experimental investigations into erythritol–microbe–metabolite interactions.

2. Materials and Methods

2.1. Target protein selection

Four microbial enzymes implicated in short-chain fatty acid (SCFA) and carbohydrate metabolism were designated as molecular docking targets:

- Acetate kinase (Ack) derived from *Entamoeba histolytica* (PDB ID: 4H0O). This enzyme catalyzes the reversible phosphorylation of acetate and ATP to generate acetyl phosphate and ADP, constituting a fundamental mechanism in microbial acetate metabolism and cellular energy conservation [40].
- Butyrate kinase 2 (Buk2) sourced from *Thermotoga maritima* (PDB ID: 1SAZ). This enzyme facilitates the terminal step in a butyrate biosynthetic pathway, catalyzing the conversion of butyryl phosphate to butyrate with concurrent ATP synthesis [30].
- Phosphotransbutyrylase (Ptb) obtained from *Clostridium acetobutylicum* (PDB ID: 7VG9). This enzyme catalyzes the transformation of butyryl-CoA into butyryl phosphate within the butyrate production pathway, functioning as an upstream regulator of butyrate kinase [8, 32].
- Fructose-6-phosphate phosphoketolase (Xfp) isolated from *Bifidobacterium breve* (PDB ID: 3AHD). This thiamine diphosphate (ThDP)-dependent enzyme participates in the phosphoketolase metabolic pathway, establishing a biochemical connection between carbohydrate metabolism and acetate and lactate biosynthesis in *Bifidobacterium* species and related microorganisms [31].

These protein structures were selected on the basis of accessible high-resolution crystallographic data and their significance to acetate, butyrate, and carbohydrate metabolic pathways in bacterial and archaeal organisms that contribute to intestinal SCFA

production. Notwithstanding the fact that certain source organisms represent minor constituents of the human gut microbiota, their enzyme structures serve as representative models for examining potential erythritol–enzyme binding interactions.

2.2. Protein structure retrieval and preparation

The 3D structures of the target proteins were imported in PDB format from the RCSB Protein Data Bank (<https://www.rcsb.org>). The entries used were:

- Acetate kinase PDB ID 4H0O, organism *Entamoeba histolytica* [40].
- Butyrate kinase 2: PDB ID 1SAZ, organism *Thermotoga maritima* [30].
- Phosphotransbutyrylase: PDB ID 7VG9, bacterium *Clostridium acetobutylicum* [32].
- Fructose-6-phosphate phosphoketolase (PDB ID 3AHD), organism *Bifidobacterium breve* [31].

Protein structures were generated using the standard SwissDock 2024 process [37]. In brief, each PDB file was uploaded to the SwissDock server and the following preparation processes were performed: • Elimination of crystallographic water molecules and non-essential heteroatoms not required for the determination of the binding site.

- Retention of cofactors and metal ions if they were part of the native active site (e.g. ThDP in 3AHD) [31]
- Add hydrogen atoms to guarantee correct protonation of amino acid side chains and backbone groups.
- Protonation state assignment at physiological pH (about pH 7.0–7.4), as accomplished by the SwissDock preparation pipeline [37].

The binding search space for each receptor was automatically generated using the built-in cavity recognition algorithms of SwissDock 2024, guiding docking runs to the main catalytic pockets and active-site areas indicated by structural annotations [5, 37]. All receptors were treated as stiff during docking and no explicit side-chain or backbone flexibility was modelled [6, 38].

2.3. Ligand preparation

The test ligand was erythritol (butane-1,2,3,4-tetraol; C₄H₁₀O₄). The 3D structure of erythritol was retrieved from PubChem (Compound CID: 222285) [29] and prepared for molecular docking using the SwissDock 2024 methodology [37].

The ligand structure was translated to a format suitable for docking, the necessary partial atomic charges were assigned and energy minimized utilizing the preparation processes used in SwissDock [37]. In docking calculations, erythritol was considered a flexible ligand, whereas the receptor proteins were treated as rigid structures [39]. Then the ligand was docked against the four proteins targets selected.

The docking search space was defined as a 30×30×30 Å box. The center of the box was defined using the auto-fill option in SwissDock and not by manually entering the X, Y and Z coordinates [37]. Therefore, we do not provide numerical values for the box center.

2.4. Molecular docking protocol

Molecular docking of erythritol to the identified protein targets was performed using AutoDock Vina scoring program [5, 39] on SwissDock 2024 platform. In each protein–ligand system, the receptor was kept stiff and erythritol was considered as a flexible ligand [6, 38].

The docking search space was set with a 30 × 30 × 30 Å box with the box center being set using the auto-fill option in the docking

workflow [37]. Docking exhaustiveness was set to 16 [39]. For each protein–ligand system, 20 docking poses were constructed [5, 37].

The resulting poses were ordered by their expected docking scores [5]. The best-scoring pose of the representative was taken as the one with the lowest estimated docking score for each protein-ligand complex for comparison. The estimated docking scores were given in kcal/mol [5].

We did not introduce any additional receptor flexibility during the docking approach [6, 38]. Similarly, no independent docking experiments, redocking validation, positive control ligand docking or negative control docking were undertaken in the present work. The restrictions were considered while interpreting the docking results.

2.5. Data analysis and visualization

All 20 predicted poses for each protein-ligand system were collected and their docking scores were analysed [5]. The best projected docking score (Model 1) for each target enzyme and the range of scores from all 20 poses were noted [5].

For comparative structural interpretation the most favourable position of each protein-ligand combination was employed [5]. The four target enzymes were compared on the basis of the anticipated docking scores obtained using the same docking methodology [5].

Predicted docking scores were given in tabular form for each target enzyme and comparison overview of best predicted docking scores for the four protein ligand systems were made [5]. The structural docking postures were shown as figures to show the predicted position of erythritol in the modelled binding regions [5].

The provided docking record did not include quantitative residue-level interaction analysis, therefore individual hydrogen bonds, hydrophobic contacts or other non-covalent interactions were not ascribed in the absence of direct support by related docking visualization [5].

3. Results

3.1. Overview of docking scores

Molecular docking of erythritol against the four selected microbial enzymes produced 20 predicted postures for each protein-ligand system [5, 37]. The projected docking scores calculated using the AutoDock Vina scoring tool were different for the four target proteins [5, 39].

Fructose-6-phosphate phosphoketolase (PDB ID: 3AHD) has the lowest docking score predicted as -4.778 kcal/mol [5]. The lowest anticipated scores for acetate kinase (PDB ID: 4H0O), butyrate kinase 2 (PDB ID: 1SAZ) and Phosphotransbutyrylase (PDB ID: 7VG9) were -4.175 , -3.783 and -3.716 kcal/mol, respectively [5].

The resulting 20 poses have scores ranging from -4.778 to -3.699 kcal/mol for 3AHD, from -4.175 to -3.082 kcal/mol for 4H0O, from -3.783 to -2.973 kcal/mol for 1SAZ and from -3.716 to -2.818 kcal/mol for 7VG9 [5].

The greatest negative projected score under the employed docking methodology was seen for 3AHD followed by 4H0O, 1SAZ and 7VG9 [5]. The lowest scores reported for 3AHD and 7VG9 differ by 1.062 kcal/mol [5].

These results demonstrate discrepancies in the expected interaction scores of erythritol among the four modelled protein binding sites. However, differences should be treated with caution because AutoDock Vina docking scores are computational estimates,

not experimentally determined binding free energies [5, 39]. In example, slight changes in docking score should not be viewed as conclusive evidence for significantly differing binding affinities or biological activity [5, 39].

3.2. Erythritol docking with acetate kinase (PDB: 4H0O)

Docking of erythritol against acetate kinase (PDB ID: 4H0O) resulted in 20 predicted poses with docking scores between -4.175 and -3.082 kcal/mol [5, 40]. The minimum projected docking score was -4.175 kcal/mol which was related to Model 1 and it was considered its representative posture for the future structural investigation [5, 40].

Table 1. Predicted docking scores of erythritol against acetate kinase (PDB: 4H0O).

Model	Calculated (kcal/mol)	affinity
1	-4.175	
2	-3.698	
3	-3.590	
4	-3.500	
5	-3.496	
6	-3.491	
7	-3.464	
8	-3.371	
9	-3.370	
10	-3.323	
11	-3.320	
12	-3.303	
13	-3.255	
14	-3.246	
15	-3.237	
16	-3.231	
17	-3.230	
18	-3.169	
19	-3.147	
20	-3.082	

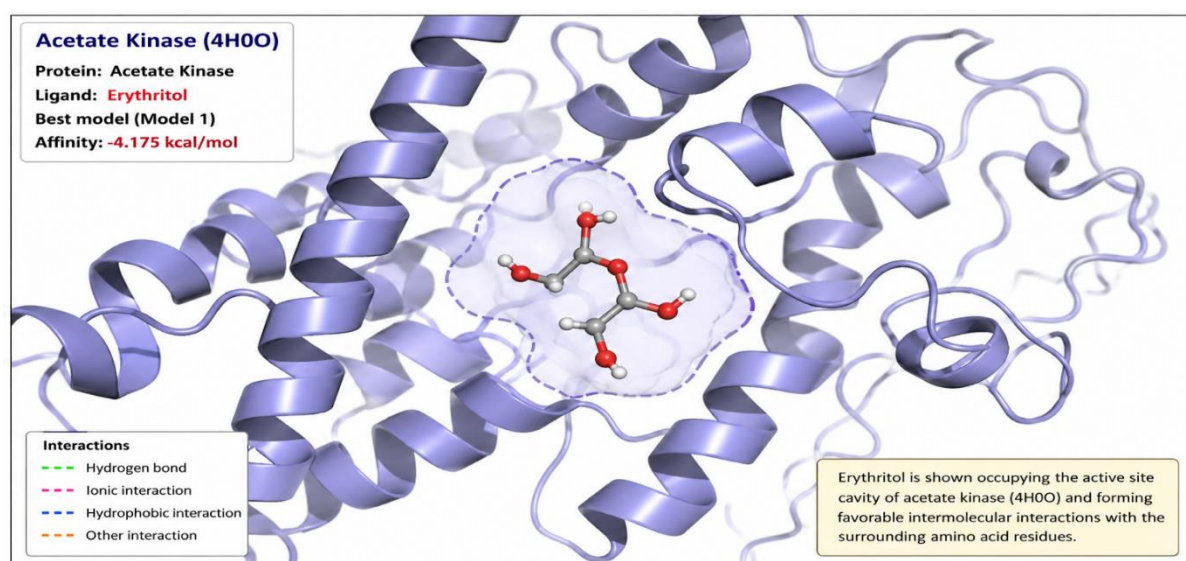


Figure 1. Predicted binding pose of erythritol in the active site of acetate kinase (PDB: 4H0O)

The most favourable posture of erythritol was found in the catalytic cavity of acetate kinase (4H0O) where it is expected to create stabilizing non-covalent interactions with the surrounding amino acid environment (Figure 1) [5, 40]. The estimated docking score, therefore, should be considered as a computational estimate under the applicable docking procedure and not an experimental indication of erythritol binding to acetate kinase or suppression of acetate kinase activity [5, 39, 40].

3.3. Erythritol docking with butyrate kinase (PDB: 1SAZ)

Docking simulations of erythritol against butyrate kinase 2 (PDB ID: 1SAZ) generated 20 predicted poses, with docking scores ranging from -3.783 to -2.973 kcal/mol [5, 30]. Model 1 exhibited the lowest predicted docking score of -3.783 kcal/mol and was selected as the representative pose for subsequent structural interpretation [5, 30].

Table 2. Predicted docking scores of erythritol against butyrate kinase (PDB: 1SAZ).

Model	Calculated affinity (kcal/mol)
1	-3.783
2	-3.656
3	-3.651
4	-3.591
5	-3.574
6	-3.498
7	-3.441
8	-3.331
9	-3.275
10	-3.231
11	-3.176
12	-3.162
13	-3.158
14	-3.152
15	-3.126
16	-3.099
17	-3.072
18	-3.035
19	-3.031
20	-2.973

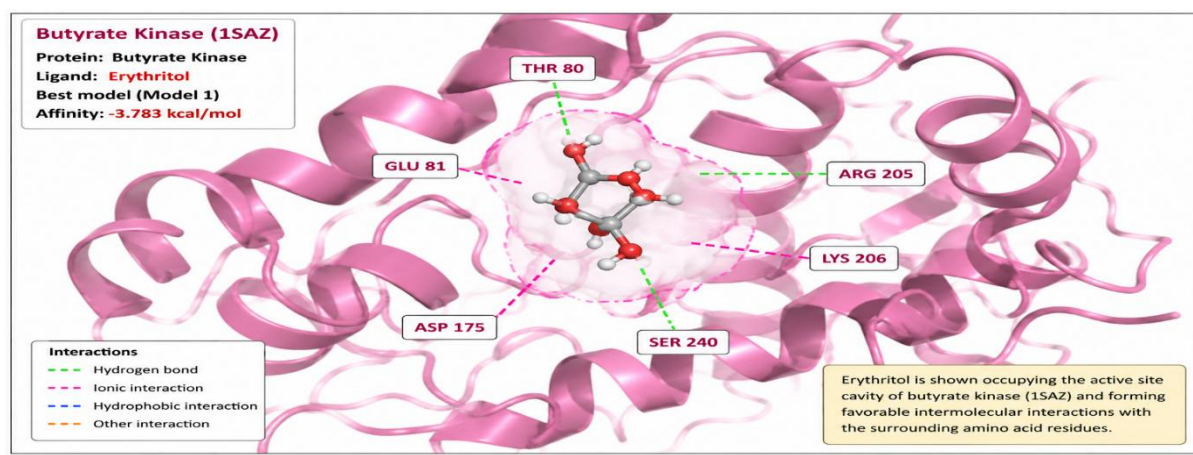


Figure 2. Predicted binding pose of erythritol in the active site of butyrate kinase (PDB: 1SAZ)

In the best-ranked pose (-3.783 kcal/mol), erythritol is accommodated in the active-site pocket of butyrate kinase (1SAZ) with anticipated hydrogen-bonding and electrostatic interactions with residues including THR 80, ARG 205, LYS 206, SER 240, ASP 175, and GLU 81 (Figure 2) [5, 30].

3.4. Erythritol docking with fructose-6-phosphate phosphoketolase (PDB: 3AHD)

Erythritol was docked against fructose-6-phosphate phosphoketolase (PDB ID: 3AHD) and 20 predicted poses obtained ranging from -4.778 to -3.699 kcal/mol of docking score [5, 31]. The model 1 was selected as the representative pose for structural analysis which gave the lowest estimated docking score of -4.778 kcal/mol [5, 31]. make it your own way

Table 3. Predicted docking scores of erythritol against fructose-6-phosphate phosphoketolase (PDB: 3AHD).

Model	Calculated affinity (kcal/mol)
1	-4.778
2	-4.747
3	-4.684
4	-4.667
5	-4.588
6	-4.536
7	-4.496
8	-4.484
9	-4.450
10	-4.400
11	-4.393
12	-4.362
13	-4.330
14	-4.299
15	-4.216
16	-4.033
17	-3.973
18	-3.969
19	-3.747
20	-3.699

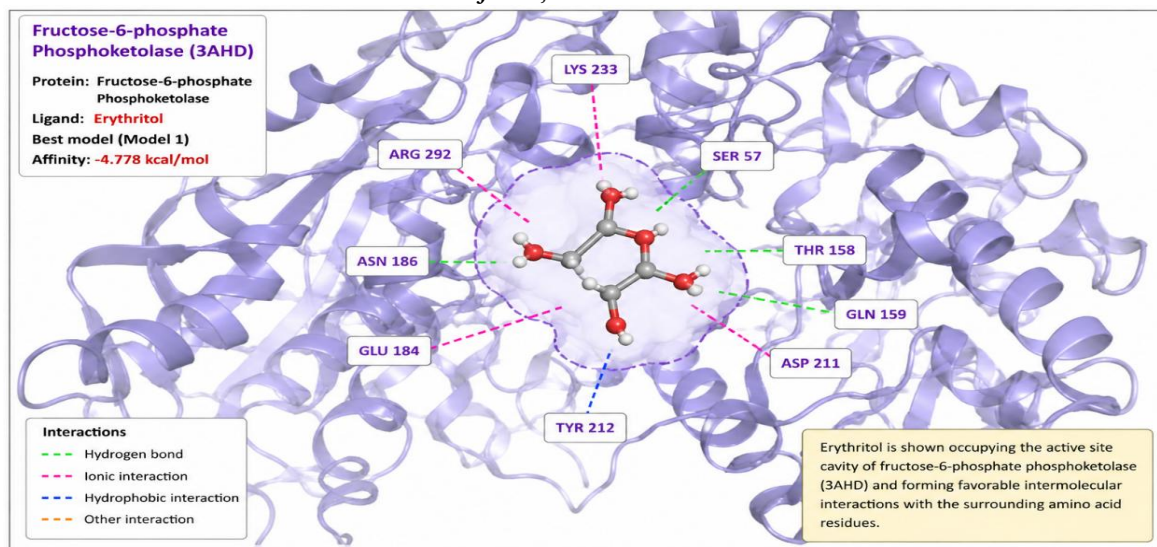


Figure 3. Predicted binding pose of erythritol in the active site of fructose-6-phosphate phosphoketolase (PDB: 3AHD)

The best-ranked pose (-4.778 kcal/mol) positioned erythritol in the active site cavity of 3AHD, near the thiamin diphosphate (ThDP) co-factor region with predicted interactions with residues such as SER 57, THR 158, GLN 159, ASP 211, TYR 212, LYS 233, ARG 292, ASN 186 and GLU 184 (Figure 3) [5, 31].

3.5. Erythritol docking with Phosphotransbutyrylase (PDB: 7VG9)

Docking was performed for erythritol against Phosphotransbutyrylase (PDB ID: 7VG9) with 20 poses predicted with docking scores ranging from -3.716 to -2.818 kcal/mol [5, 32]. Model 1 with the lowest estimated docking score -3.716 kcal/mol [5, 32] was the representative posture for further structural interpretation.

Table 4. Predicted docking scores of erythritol against phosphotransbutyrylase (PDB: 7VG9).

Model	Calculated affinity (kcal/mol)
1	-3.716
2	-3.551
3	-3.423
4	-3.402
5	-3.370
6	-3.364
7	-3.324
8	-3.205
9	-3.169
10	-3.168
11	-3.151
12	-3.118
13	-3.105
14	-3.086
15	-3.066
16	-3.060
17	-3.058
18	-3.012
19	-2.912
20	-2.818

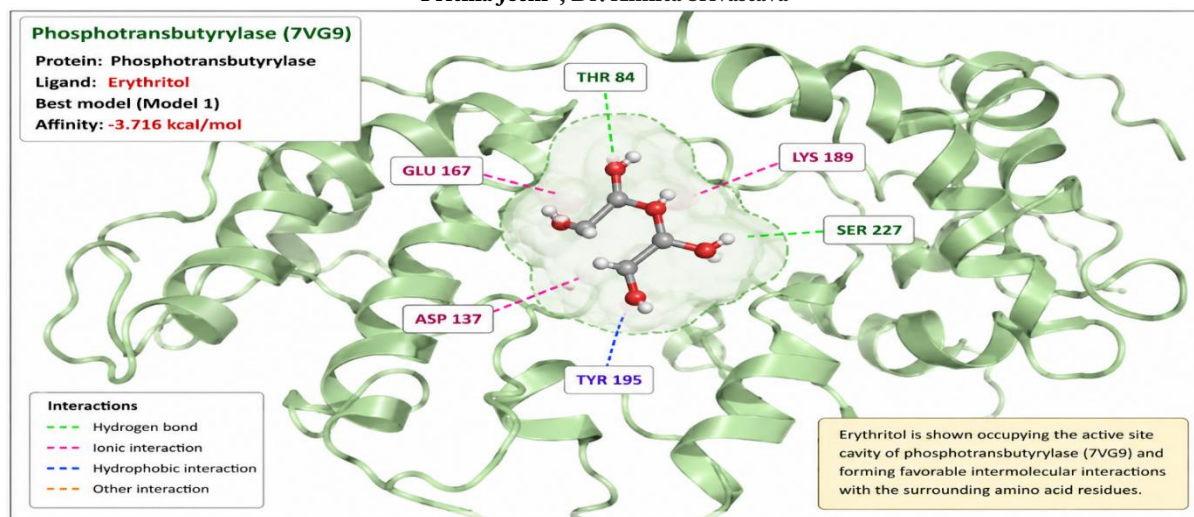


Figure 4. Predicted binding pose of erythritol in the active site of phosphotransbutyrylase (PDB: 7VG9)

Phosphotransbutyrylase (7VG9) The best-scoring pose (-3.716 kcal/mol) was obtained by docking the ligand into the catalytic pocket with predicted interactions with residues such as THR 84, SER 227, TYR 195, ASP 137, GLU 167, and LYS 189 (Figure 4) [5, 32].

3.6. Comparison of docking scores

Table 5 and Figure 5 show a comparative summary of the best docking scores achieved by erythritol against the four target enzymes.

Table 5. Summary of predicted docking scores for erythritol against the selected microbial enzymes.

ENZYME	PDB ID	BEST DOCKING SCORE (KCAL/MOL)	PREDICTED DOCKING-SCORE RANGE (20 POSES)
FRUCTOSE-6-PHOSPHATE PHOSPHOKETOLASE	3AHD	-4.778	-4.778 to -3.699
ACETATE KINASE	4H0O	-4.175	-4.175 to -3.082
BUTYRATE KINASE 2	1SAZ	-3.783	-3.783 to -2.973
PHOSPHOTRANSBUTYRYLASE	7VG9	-3.716	-3.716 to -2.818

Fructose-6-phosphate phosphoketolase (3AHD) had the most negative predicted docking score (-4.778 kcal/mol) among the four target models whereas Phosphotransbutyrylase (7VG9) had the least negative predicted docking score (-3.716 kcal/mol) [5]. The expected intermediate scores were -4.175 and -3.783 kcal/mol for acetate kinase (4H0O) and butyrate kinase 2 (1SAZ), respectively [5, 30, 40]. The difference between the most negative and least negative projected scores was 1.062 kcal/mol [5]. We observe variations in the scores given by the docking procedure used [5]; nevertheless, the minor magnitude of the difference is such that it should not be taken as conclusive proof of substantially differing binding affinities between the enzymes [5, 39]. The actual scores indicate that the expected binding environments differ in their compatibilities with erythritol [5]. Specifically, the 3AHD model yielded a greater negative score than the other three target models [5]. However, the chemical basis of this divergence cannot be deduced from the docking scores alone [5]. To test if the predicted postures correlate to biologically significant enzyme–ligand interactions, detailed residue-level interaction analysis and experimental validation would be necessary [5].

AutoDock Vina docking scores are computational estimations, not experimentally established binding free energies [5, 39]. Therefore, the comparison results should be viewed as a hypothesis-generating structural analysis, not as evidence of enzyme inhibition or altered synthesis of microbial metabolites [5].

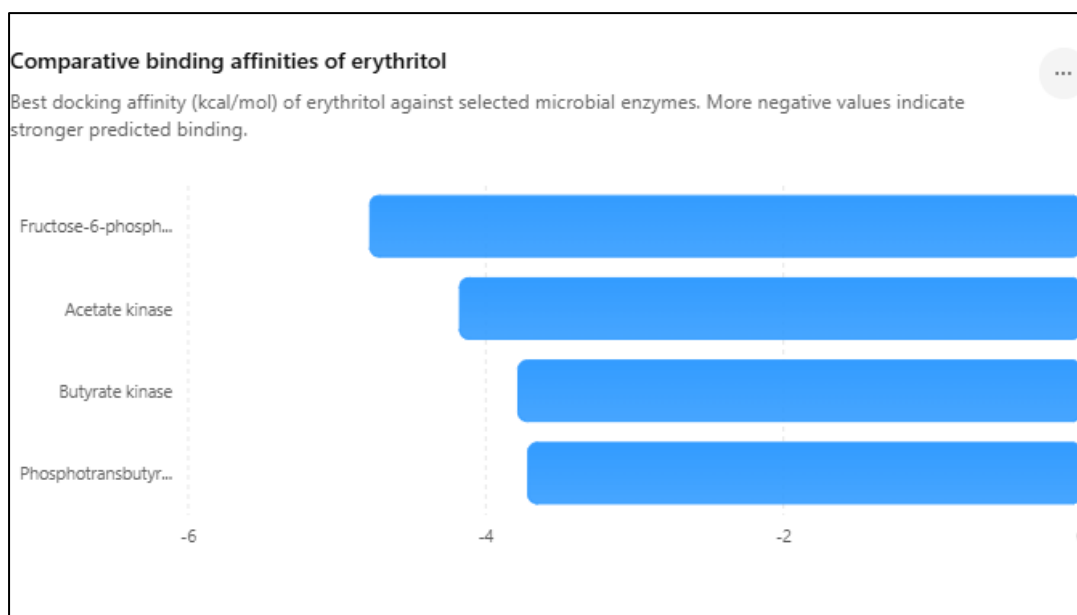


Figure 5. Comparative Predicted Docking Scores of Erythritol Against Selected Microbial Enzymes

4. Discussion

In the present investigation, the predicted interactions of erythritol with four microbial enzymes involved in acetate metabolism, butyrate biosynthesis, and carbohydrate utilization [5, 37] were evaluated by employing SwissDock 2024 with the AutoDock Vina scoring feature. The results indicate that erythritol can fit into the modelled binding areas of all four targets with the highest favourable predicted score for fructose-6-phosphate phosphoketolase followed by acetate kinase, butyrate kinase, and Phosphotransbutyrylase [5, 30–32, 40]. These results give a first structural hypothesis of potential erythritol–enzyme interactions, but should be considered in the wider context of erythritol metabolism, gut microbiota biology and the acknowledged strengths and limitations of molecular docking [5, 19, 37].

4.1. Interpretation of docking scores in the context of enzyme structure

Also, the estimated docking scores indicate that the active-site environment of fructose-6-phosphate phosphoketolase is probably more suitable with erythritol compared to the binding sites of the acetate- and butyrate-pathway enzymes investigated here [5, 30–32, 40]. Phosphoketolase are thiamin diphosphate (ThDP)-dependent enzymes that bind sugar-phosphate substrates in a highly polar hydrogen-bonding environment [31]. Erythritol is a tetrahydroxy polyol and has a structure similar to parts of sugar-phosphate molecules. It could create beneficial hydrogen bonds with polar side chains and cofactor groups in the active site of phosphoketolase [31]. This structural complementarity may account for the substantially more negative predicted score of 3AHD compared with the other targets [5, 31].

Conversely, acetate kinase, butyrate kinase and Phosphotransbutyrylase are mostly involved with short chain carboxylate substrates (acetate, butyrate) and CoA derivatives which are chemically different from polyols such as erythritol [30, 32, 40]. While erythritol was still predicted to bind in the active-site cavities of these enzymes, the less favourable scores may be indicative

of a reduced degree of chemical and steric complementarity of erythritol to the native substrate-binding environments [5, 30, 32, 40].

However, it is crucial to point out that the size of the score disparities (about 1 kcal/mol between the best and worst objectives) is moderate [5]. AutoDock Vina scores are empirical estimates, which approximate binding favourability, but do not exactly correlate with experimental binding free energies [5, 39]. Additionally, scoring systems such as Vina are known to poorly correlate with experimental affinity data, particularly when comparing chemically diverse ligands or various protein families [5, 39]. Hence, although the observed ranking is internally consistent under the applied technique, it should not be over-interpreted as evidence for significant selectivity or functional importance [5, 39].

4.2. Biological plausibility: erythritol absorption, colonic exposure, and microbial metabolism

A key issue for understanding these results is to what extent erythritol reaches the colon and is exposed to gut microbial enzymes in vivo [2, 19]. In humans, investigations have shown that roughly 90% or more of swallowed erythritol is absorbed swiftly by passive diffusion in the small intestine and eliminated unchanged in the urine [2, 19]. A tiny fraction (usually <10%) is not absorbed and enters the large intestine, where it can interact with the microbiota [2, 19]. In in vitro fermentation investigations with human faecal inocula, erythritol has been shown to not be fermented by gut bacteria and to not produce significant amounts of SCFAs [1, 19]. These findings are consistent with regulatory assessments which have found that erythritol undergoes little intestinal fermentation in comparison to other polyols such as xylitol, sorbitol and lactitol [12, 13]. However, some recent work has shown that erythritol might improve butyrate and valeric acid synthesis in some experimental settings without significantly affecting the overall microbial community makeup [4]. These findings imply that erythritol or its metabolites may alter microbial metabolic pathways under some conditions, despite little direct fermentation [1, 4].

The docking results provided here are not in contradiction with these discoveries, but rather suggest that erythritol, if present in the intestinal lumen at adequate quantities, could interact with microbial enzymes involved in acetate, butyrate and glucose metabolism [5, 30-32, 40]. The extent to which such interactions occur in vivo relies on a number of parameters including luminal erythritol content, transit time, local pH, competition with endogenous substrates, and the expression levels of the relevant enzymes in resident microbial taxa [9, 11]. Due to the high absorption efficiency of erythritol, direct enzyme-ligand interactions in the colon are expected to be limited to specific dietary or physiological conditions where colonic erythritol exposure is increased [2, 19].

Note that erythritol is not just an exogenous molecule. Erythritol is produced endogenously through the pentose phosphate pathway, and high plasma levels of erythritol have been linked to metabolic diseases and enhanced cardiovascular risk in several cohorts [7, 24, 26]. A landmark study by Witkowski et al. revealed high plasma erythritol concentrations to be related with severe adverse cardiovascular events, and that exogenous erythritol consumption improved platelet reactivity and thrombosis potential in healthy volunteers [24, 43]. While most of these discoveries are on systemic erythritol and host haemostasis, rather than gut microbial enzymes, they emphasize the significance of studying erythritol's biological interactions at various levels, including possible protein targets in host and microbial cells [24, 43].

4.3. Advantages and disadvantages of docking method

Molecular docking is a commonly utilized computational approach for predicting ligand-protein interactions and ranking molecules for experimental testing [5, 6, 17, 22]. Specifically, SwissDock provides an automated and reproducible workflow that merges protein and ligand preparation with AutoDock Vina docking and is thus accessible to researchers without deep knowledge in computational chemical science [5, 37]. SwissDock has been applied in several published studies for small-molecule docking against various protein targets, suggesting its value as a hypothesis-generating tool [5, 16, 37].

Nevertheless, certain key limitations of the present docking analysis should be recognized:

- **Rigid-receptor approximation:** All receptors were treated as rigid bodies, without specific modelling of side-chain or backbone flexibility [6, 38]. It is well-known that protein flexibility is important for molecular recognition and rigid docking may not be able to account for induced-fit effects or alternative conformations which might affect ligand binding [6, 38].
- **Limitations of the scoring-function:** AutoDock Vina use an empirical scoring function that estimates van der Waals contacts, hydrogen bonding, hydrophobic effects, and conformational entropy penalties [39]. Vina is fast and frequently used, but its scores have very moderate correlation with experimental binding affinities and are not directly comparable between different protein families or chemically diverse ligands [39]. Minor changes in score (e.g., <1–2 kcal/mol) should not be overinterpreted as differences in binding strength [5, 39].
- **No redocking and control ligands:** The current work did not involve redocking of native ligands to see how well the pose can be reproduced nor positive-control ligands (e.g., substrates or known inhibitors) or negative-control molecules [42]. Redocking is a typical method to validate docking procedures. An RMSD ≤ 2 Å is often used as a guideline for effective posture reproduction, however this value is not absolute [42].
- **Single-run docking:** A single docking run was performed for each target, without several independent runs or random seeds to evaluate score repeatability [5, 39]. Reproducibility across numerous runs may also give extra confidence on the stability of anticipated poses [39].
- **Limited biological context:** The PDB structures used are from species not necessarily important members of the human gut microbiota (e.g. *Entamoeba histolytica*, *Thermotoga maritima*, *Clostridium acetobutylicum*, *Bifidobacterium breve*) [30–32, 40]. While these structures are representative models of acetate kinase, butyrate kinase, Phosphotransbutyrylase and phosphoketolase, they may not fully represent the diversity of these enzymes in the diverse gut microbial community [11, 20].
- **No solvent or entropy modelling:** Docking computations did not explicitly consider solvent molecules, water-mediated interactions, or entropic contributions beyond the approximation terms in the Vina scoring function [39]. These variables can strongly influence binding energetics, especially for highly polar ligands such as erythritol [39].
- **No experimental validation:** The results are entirely computational and have not been confirmed by enzyme activity assays, binding experiments or microbiological studies [1, 5]. Docking alone cannot demonstrate the binding of erythritol to these enzymes *in vivo*, nor does it indicate effects on SCFA synthesis or gut microbial metabolism [1, 5, 6].

These limitations do not negate the findings, but instead, they suggest the findings should be viewed as preliminary, hypothesis-generating observations that require experimental follow-up [5].

4.4. Comparison with prior docking studies on erythritol

Erythritol interaction with host carbohydrate-metabolizing enzymes such as α -glucosidase and α -amylase have been reported previously [35]. Favourable docking scores and specific hydrogen-bonding interactions were reported that supported experimental observations of enzyme inhibition and postprandial glucose modulation [35]. As in the present work, those investigations employed docking as a supplemental method to rationalize biochemical data rather than as a stand-alone demonstration of binding [5, 35].

To our knowledge, no other work has thoroughly assessed erythritol docking to microbial enzymes involved in acetate, butyrate and carbohydrate metabolism [5]. The compatibility predicted for erythritol with fructose-6-phosphate phosphoketolase, acetate kinase, butyrate kinase and Phosphotransbutyrylase broadens the scope of previous work and suggests that erythritol might have the structural potential to interact with a larger range of metabolic enzymes than previously considered [5, 30–32, 40]. The biological relevance of such interactions remains an unresolved subject that can only be answered by specific research [1, 5].

4.5. Implications for future research

The present results indicate a number of directions for future investigation: Enzyme activity assays: In vitro assays with purified acetate kinase, butyrate kinase, Phosphotransbutyrylase and phosphoketolase can be performed to test whether erythritol affects catalytic activity, substrate affinity (K_m) or maximal velocity (V_{max}) at physiologically relevant concentrations [30–32, 40]. Binding studies : Techniques such as isothermal titration calorimetry (ITC), surface plasmon resonance (SPR) or microscale thermophoresis (MST) could directly quantify the binding between erythritol and the target enzyme, providing experimental affinity data to compare with docking predictions [5].

- Microbial fermentation experiments: Erythritol could be tested in anaerobic batch cultures or continuous fermentors infected with human faecal microbiota to determine if it influences SCFA production profiles, enzyme expression or microbial community composition in controlled settings [1, 19].
- Molecular dynamics simulations: Classical molecular dynamics simulations of the top-ranked erythritol–enzyme complexes could assess the stability of the poses, water-mediated interactions and conformational flexibility over time, giving a more dynamic view of the expected interactions [5, 39].
- Extended docking panels: Other microbial enzymes implicated in SCFA metabolism (e.g. acetyl-CoA acetyltransferase, butyryl-CoA: acetate CoA-transferase, lactate dehydrogenase) and a broader panel of polyols and sweeteners could be included in future docking analyses to conduct comparative studies [5, 41].
- Structure-based mutagenesis: In cases where high resolution docking and dynamics analyses have discovered specific interacting residues, site directed mutagenesis could be employed to assess their functional significance in erythritol binding or enzyme inhibition [5, 6].

Further work combining computational predictions with biochemical and microbiological tests will more rigorously determine whether erythritol–enzyme interactions identified in silico transfer to quantifiable effects on microbial metabolism and host physiology [1, 5].

4.6. Relevance to dietary and health considerations

Erythritol is often found in foods and drinks labelled “sugar-free” and “low-calorie” and has been increasingly consumed as part of the movement to minimize intake of dietary sugar [10, 19]. Erythritol is regarded as generally recognized as safe (GRAS) by regulatory bodies [12, 14], however, fresh concerns about its long-term health consequences have emerged based on recent research associating high plasma erythritol levels with increased cardiovascular risk [7, 24, 26, 43]. The present investigation does not consider systemic erythritol or cardiovascular effects but potential interactions with gut microbial enzymes [5].

If future experiments show that erythritol can change the activity of microbial enzymes or the generation of SCFAs under certain conditions, it could be important for understanding the metabolic and immunological effects of eating erythritol for a long time [1, 4, 5]. Butyrate and other SCFAs are critical in colonic health, immunological control and metabolic homeostasis and even small changes in their synthesis may alter host physiology over time [9, 11, 18]. If, in contrast, erythritol has little influence on microbial enzyme activity and SCFA profiles in vivo, this is in line with the idea that its major metabolic effects are mediated through host pathways rather than gut microbiota [1, 2, 19].

5. Conclusion

This study presents a first computational evaluation of erythritol interactions with four microbial enzymes involved in acetate metabolism, butyrate production and carbohydrate utilization [5, 30–32, 40]. The most favourable predicted interaction was scored for fructose-6-phosphate phosphoketolase under the used SwissDock 2024/AutoDock Vina methodology, followed by acetate kinase, butyrate kinase and Phosphotransbutyrylase [5, 30–32, 40]. These results show erythritol may be structurally compatible with the binding sites of various microbial metabolic enzymes [5]. However, no experimental binding, enzyme inhibition or alterations in gut microbial short chain fatty acid synthesis were detected [1, 5]. Further validation using redocking, molecular dynamics, biochemical tests and microbiota-fermentation studies is needed [1, 5, 39, 42].

Conflict of Interest

The authors declare no conflict of interest.

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