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Bacteriolytic activity of lactoferrin: its modulation by small molecules and synergy with lysozyme

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Research Article

Keywords: bacteriolytic activity, lactoferrin, lysozyme, Escherichia coli, peptide semax

Posted Date: November 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11604361/v1

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Additional Declarations: No competing interests reported.

Abstract

This study demonstrates the bacteriolytic activity of human lactoferrin on intact cells. We investigated the effects of pH, ionic strength, and low-molecular-weight compounds – including amino acids, oligopeptides, and the nonionic surfactant Tween-21 – in modulating this lytic activity. The combined bacteriolytic action of lactoferrin and lysozyme was also investigated. Charged amino acids (Arg, His, Glu) and Gly at 1–5 mM enhanced LTF-mediated lysis 2.2- to 4.5-fold. The non-ionic surfactant Tween-21 (0.1–0.2 μM) enhanced LTF activity 3.2-fold. A panel of structurally diverse oligopeptides and small molecules – selected based on their charge, conformation, and amphipathic profiles – was evaluated for LTF-modulating activity. Antimicrobial and regulatory peptides – including bacitracin, the synthetic hexapeptide imunofan, and the heptapeptide semax (MEHFPGP) – at micromolar concentrations synergistically amplified LTF lytic activity up to 12-fold. The cyclic peptide gramicidin S, the macrocyclic lactone ivermectin, and the regulatory peptides dalargin and selank exhibited intrinsic bacteriolytic activity against , but they did not enhance bacteriolytic activity of LTF. LTF and lysozyme act synergistically: their combined lytic rate is twofold higher than the calculated additive effect, suggesting a sequential or cooperative disruption of the cell envelope. These findings expand the understanding of LTF as a direct lytic agent and elucidate that small-molecule effectors can modulate the physiological efficacy of cationic host-defense proteins.

Introduction

Bacterial resistance to antibiotics has become a critical global healthcare challenge [1]. The rapid proliferation rate of bacterial cells underlies their capacity for extensive mutagenesis and high genotypic variability. Under selective pressure, the rapid elimination of susceptible cells facilitates the emergence of adaptive genotypes. This enables bacterial populations to withstand existing antibiotics through diverse protective mechanisms, including the production of specific enzymes (e.g., penicillinases, beta-lactamases, and carbapenemases), ribosomal protection proteins (such as TetM), PBP2a modifications, terminal target alterations (e.g., the replacement of D-Ala-D-Ala with D-Ala-D-Lac mediated by vanA/B genes), and active efflux systems (such as TetA transport proteins) [2–5]. Consequently, conventional pipelines for the development, screening, preclinical and clinical testing of low-molecular-weight synthetic and semi-synthetic drugs are failing to keep pace with rapidly evolving microorganisms.

Therefore, the use of native high-molecular-weight antimicrobial compounds (proteins and enzymes), as well as various compositions based on them, including those with added low-molecular-weight effectors, is of great importance to combat bacterial infections. The deployment of native macromolecules offers several distinct advantages, including reduced drug development timelines, a low propensity for bacterial resistance, high biocompatibility, minimal adverse reactions, and cost-effectiveness. Furthermore, native macromolecules effectively target persister cells [6], whereas conventional antibiotics predominantly act against actively dividing cells via specific mechanisms: disrupting cell wall synthesis (penicillins, cephalosporins, carbapenems [7]), inhibiting protein synthesis through ribosome binding (macrolides, tetracyclines, aminoglycosides [8]), or impairing transcription and DNA replication via topoisomerase

inhibition (fluoroquinolones [9]). Finally, certain native macromolecules exhibit nonspecific modes of action based on the most general biochemical principles of bacterial cell envelope structure, which, in turn, are determined by fundamental physical laws. Consequently, despite their high evolutionary potential, bacterial cells rarely develop resistance to such agents as lysozyme, which hydrolyzes the peptidoglycan murein of the bacterial cell wall, or defensins, which disrupt the membrane via electrostatic and hydrophobic interactions [10].

Lactoferrin (LTF) represents one such antimicrobial agent; it is a multifunctional protein [11, 12] constitutively present in various mammalian tissues and secretions [13]. Diverse biological properties of LTF have been characterized: iron binding and retention [14], antimicrobial [15] and antiviral [16] actions, immunomodulation [17, 18], gene expression modulation [19], as well as proteolytic [20] and nuclease [21] functions. Historically, the antibacterial efficacy of LTF has been predominantly attributed to its capacity to sequester iron ions, thereby depriving microorganisms of an essential growth element – a classical bacteriostatic mechanism [22]. Conversely, the direct bacteriolytic properties of LTF, which involve the immediate disruption of intact bacterial cells, remain uncharacterized.

Structurally, LTF belongs to the transferrin superfamily. Human plasma serotransferrin, another member of this family, has been demonstrated to lyse several bacterial species, including *E. coli*, *Micrococcus luteus*, *Bacillus subtilis*, and *Priestia megaterium* (*Bacillus megaterium*) [23]. Notably, LTF is a highly cationic protein; its surface-exposed positively charged amino acid residues outnumber negatively charged ones by 18–24%, contingent upon iron saturation and the degree of glycosylation [23]. Additionally, the presence of several surface cationic clusters – areas characterized by a compact arrangement of positively charged residues – has been shown [23] to be a key factor determining the protein's ability to interact with bacterial cells and subsequently disrupt their membrane integrity. In our study, we assumed that these properties may mediate the interaction of the LTF molecule with the negatively charged *E. coli* cell surface, which is driven by phosphate groups and lipopolysaccharides.

Previously, we demonstrated that charged amino acids, acting as low-molecular-weight additives, enhance the bacteriolytic activity of lysozyme [24]. Therefore, the present study evaluates whether such additives can similarly enhance the activity of LTF. Additionally, polysorbate Tween-21 (ethoxylated (4) sorbitan monolaurate) was evaluated as another potential low-molecular-weight effector of LTF. This non-ionic surfactant is safe for the human body, widely used in the cosmetic and food industries, and has been shown to increase the activity of lysozyme at micromolar concentrations [25].

Furthermore, oligopeptides show significant promise as additives in various therapeutic formulations within medicine and biotechnology. The primary advantages of oligopeptides include biodegradability, relative ease of synthesis, and the ability to not only activate the enzyme but also enhance its stability and act as a protective shield against bacterial proteolysis. Previously, we demonstrated that low concentrations of the cyclic peptide antibiotics polymyxin and bacitracin enhance the efficiency of bacterial cell lysis by lysozyme and interleukin-2 [26]. To extend this line of investigation, we examined the effects of several cyclic and linear oligopeptides and other small molecules on lactoferrin activity.

These oligopeptides are components of various therapeutics widely used to treat diverse diseases: dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), a synthetic analogue of endogenous leucine enkephalin, used for tissue regeneration [27]; semax (Met-Glu-His-Phe-Pro-Gly-Pro), a heptapeptide with neuroprotective and nootropic properties [28]; selank (Thr-Lys-Pro-Arg-Pro-Gly-Pro), a member of the anxiolytic peptides family that enhances cognitive abilities and is utilized as an antidepressant [29]; imunofan (Arg-αAsp-Lys-Val-Tyr-Arg), a synthetic immunomodulator that additionally displaying detoxifying and hepatoprotective effects [30]; gramicidin S (*cyclo*(-L-Val-L-Orn-L-Leu-D-Phe-L-Pro-)$_2$), an antibacterial agent [31]; bacitracin (L-Thz-D-Leu-L-Ile-L-Lys(-cyclo-[-D-Orn-L-Ile-D-Phe-L-His-D-Asn-D-Asp-]), an antibacterial agent produced by *B. subtilis* [32]; ivermectin (mixture of $C_{48}H_{74}O_{14}$ (22,23-dihydroavermectin B_{1a}) and $C_{47}H_{72}O_{14}$ (22,23-dihydroavermectin B_{1b})), a macrocyclic lactone antiparasitic agent [33]. The inclusion of these compounds is strategically justified by their distinct physiochemical profiles, serving as model representatives of their respective chemical classes.

Evaluating the combined effects of LTF and bacteriolytic enzyme lysozyme is of particular interest, with a specific focus on their potential synergy. Lactoferrin and lysozyme are simultaneously present in various biological fluids, including blood plasma [34], colostrum and milk [35], respiratory secretions [36], saliva and tears [37, 38]. However, the synergistic bacteriolytic effects of these two proteins on intact bacterial cells has not yet been studied.

E. coli was selected as a lactoferrin substrate in this study because this microorganism (specifically, strain 3GCR), along with *Klebsiella pneumoniae*, is designated by the WHO as a priority drug-resistant pathogen [39]. Both *E. coli* and *K. pneumoniae* belong to the same family, *Enterobacteriaceae*. However, the *E. coli* strain KS-507 (K-12 lineage, B-3254) used in this study is opportunistic, making it a suitable model organism for these experiments.

Thus, this study investigates the bacteriolytic properties of lactoferrin against intact *E. coli* cells in the presence of low-molecular weight additives, such as charged amino acids, glycine, surfactant Tween-21 and a number of oligopeptides as potential effectors, while also evaluating the combined application of lactoferrin and lysozyme.The discovery that low-molecular-weight effectors amplify LTF activity, alongside its molecular synergy with lysozyme, provides critical insights into host-defense protein cooperation in vivo during envelope disintegration. These findings provide a framework for designing targeted formulations capable of dismantling recalcitrant bacterial cell walls and membranes.

Materials and Methods

Materials

The following reagents were used in the study: NaCl, HCl, polysorbate Tween-21, Tris, EDTA (Sigma-Aldrich, USA); sodium citrate (Reakhim, Russia); L-lysine and L-histidine (Serva, Germany), L-arginine (Merck, Germany); L-glutamic acid (MeiHua, China); glycine (Carl Roth, Germany); bacitracin (HiMedia Laboratories, India); gramicidin S (Huadong Medicine, China); imunofan (Bionix, Russia); selank, semax

(Peptogen, Russia); dalargin (Mikrogen, Russia); human lactoferrin and human lysozyme (Sigma-Aldrich, USA). Ivermectin, active pharmaceutical ingredient of the commercial formulation Ivermek (Nita-Pharm, Russia). The buffer system containing 20 mM Tris, 0.5 mM EDTA, 1 mM sodium citrate, and 40 mM NaCl (pH 7.4) was used in all experimental series (unless otherwise stated). An ExplorerPro analytical balance (Ohaus, USA) was used to weigh the reagents. The pH of the buffer solutions was adjusted using an Orion 420 + pH meter (Thermo, USA) equipped with an ESLK-01.7 electrode (Aquilon, Russia).

Cell culture and treatment conditions

E. coli strain KS-507 (K-12 lineage, B-3254) was obtained from the All-Russian Collection of Industrial Microorganisms (National Research Center "Kurchatov Institute", Russia). *E. coli* cell cultures were stored at − 70°C in a Scientific's Premium Ultra-Low Temperature (ULT) lab freezer (New Brunswick, USA) and thawed immediately prior to experiments. The cultures were centrifuged at 6,000 rpm (2,415×g) for 5 min using a MiniSpin centrifuge (Eppendorf, Germany). The supernatant was then discarded, and the cell pellet was resuspended in a buffer solution containing 20 mM Tris, 0.5 mM EDTA, 1 mM sodium citrate, and 40 mM NaCl (pH 7.4). A freshly prepared *E. coli* cell suspension was diluted to an optical absorption (OD at a wavelength of 630 nm) value of A_{630nm} = 0.30 ± 0.02, corresponding approximately to 1.6×10^8 colony-forming units per millilitre (CFU/mL). Bacteriolytic activity was measured turbidimetrically by monitoring the decrease in the OD of the cell suspension, which is proportional to the change in cell count over time (d[CFU]/dt [24]). Measurements were performed using a Multiskan plate spectrophotometer (Thermo Scientific, USA) at 630 nm and 25°C. The kinetics of enzymatic lysis were recorded in real time at 10 s intervals. To calculate the lysis rate, the background absorbance change was subtracted from the total OD decrease obtained for each sample as a correction for baseline drift.

Experimental design and optimization of assay conditions

To establish the baseline parameters, we first determined the dependence of *E. coli* lysis kinetics on LTF concentration at a physiological pH of 7.4 and a NaCl concentration of 40 mM. Subsequently, the lysis of viable *E. coli* cells was investigated at a fixed LTF concentration of 2.5 μg/mL over a wide range of pH and ionic strength values. For experiments determining the dependence of LTF activity on NaCl concentration, the salt concentration was varied from 0 to 150 mM (Fig. 1B). Screening of potential low-molecular-weight lactoferrin effectors (amino acids, Tween-21, and oligopeptides) was performed using the same LTF concentration (2.5 μg/mL) at pH of 7.4, a NaCl concentration of 40 mM, and a temperature of 25°C.

Finally, potential low-molecular-weight lactoferrin effectors (amino acids, Tween-21, and oligopeptides) were screened using the optimized LTF concentration (2.5 μg/mL) at physiological pH (7.4), 40 mM NaCl, and 25°C.

Results and Discussion

Figure 1A demonstrates the dependence of *E. coli* lysis rate on the LTF concentration at the physiological pH 7.4 and the buffer solution ionic strength of 40 mM NaCl. Analysis of the data presented in Fig. 1A allows us to conclude that *E. coli* cells are successfully lysed in the presence of LTF; the dependence of the lysis rate on the LTF concentration is essentially linear within the range of 0.0–3.0 μg/ml. Figure 1B demonstrates the dependence of *E. coli* lysis rate on pH and ionic strength at a fixed LTF concentration of 2.5 μg per mL. As we can see, the highest lysis rate values under these conditions are achieved at pH values of 7.6–8.2 and a NaCl concentration of 30–60 mM. Typically, a bell-shaped dependence with a pronounced pH optimum of activity corresponds to enzymatic catalysis with an active center [24]. However, the existence of an active center in LTF (for example, similar to the active center of lysozyme, which would specifically cleave the peptidoglycan of the cell wall) has not been described to date. If the bacteriolytic effect of lactoferrin is due exclusively to electrostatic interactions (the «cationic» effect), then the change in pH would most likely not have a narrow maximum (Fig. 1B). However, the literature does describe a limited number of examples where a catalytic agent (macromolecule) had no active site and was not strictly an enzyme (in the strict sense of the term) nevertheless had catalytic activity with a pH optimum. For example, imidazole polymers [40] or a copolymer of L-cysteine and L-glutamic acid [41]. Therefore, the possibility of the existence of a pH optimum even with the purely cationic nature of LTF action cannot be completely ruled out. Presumably, this may be due to the fact that the interaction of the protein with the bacterial cell surface under certain conditions leads to conformational changes, resulting in more accessible amino acid residues, which also interact with cell surface structures and enhance the effect, reaching maximum values at certain pH values.

At the same time, another mechanism – a catalytic one – is also possible. The proteolytic properties of LTF were described in the literature [20, 42]. In this regard, it can be hypothesized that LTF has also the ability to hydrolyze peptide bonds in murein cross-links of Gram-negative bacteria. Bacteriolytic mechanism combining cationic and hydrolase (possibly protease) activities is also possible. In this regard, a certain parallel can be drawn with lysozyme: it is capable of lysing bacterial cells by cleaving peptidoglycan via the catalytic residues Glu35 and Asp53 in the active site [43], but is also capable of acting via a cationic mechanism [44, 45]. The precise mechanism underlying LTF bacteriolytic activity remains to be determined in future studies.

The results of the screening potential low-molecular effectors of LTF are shown in Figs. 2–3 and summarized in Table 1. Figure 2 (A, B, C, D) shows that among the tested amino acids, 3 mM histidine and 2 mM glycine induced the most pronounced effects (4-fold and 3-fold increases, respectively). Moreover, significant 2.2-fold and 2.7-fold increases were observed upon treatment with 2 mM arginine and 5 mM glutamate, respectively. Lysine in the concentration range of 1–10 mM had no noticeable effect on the lysis rate. In the absence of LTF none of these amino acids effect bacterial cells.

Table 1
The effect of low-molecular-weight compounds on the lysis of *E. coli* cells in the presence and absence of lactoferrin. [LTF] = 2.5 μg per mL.

Effector	Concentration	Cell lysis by effector	Increase of cell lysis by lactoferrin, fold
Arginine	2.1 mM	no effect	2.2
Glutamate	7.0 mM	no effect	2.7
Glycin	2.1 mM	no effect	3.5
Histidine	2.8 mM	no effect	4.5
Lysine	0.0–10.0 mM	no effect	no effect
Bacitracin	5.7 μM	no effect	2.0
Imunofan	5.0 μM	no effect	4.0
Semax	0.05–0.15 μM	no effect	7.0–12.0
Tween-21	0.1–0.2 μM	no effect	3.2
Dalargin	0.1–0.6 μM	effect on cells	no effect on cell lysis by lactoferrin
Selank	0.1–1.4 μM	effect on cells	no effect on cell lysis by lactoferrin
Gramicidin S	1.0–15.0 μM	effect on cells	the simple sum of cell lysis by an effector and by lactoferrin
Ivermectin	1.0–15.0 μM	effect on cells	the simple sum of cell lysis by an effector and by lactoferrin

Figures 2 (E, F, G, H) and 3 present the results of experiments investigating *E. coli* lysis by lactoferrin in the presence of certain oligopeptides (active pharmaceutical ingredients in drugs) and the nonionic surfactant Tween-21. Among these compounds, semax (7- to 12-fold), immunofan (4-fold), and bacitracin (2-fold) significantly enhanced the bacteriolytic activity of LTF. Gramicidin S and ivermectin, on their own, act as lytic agents, destroying *E. coli* cells but do not affect bacterial lysis in the presence of LTF. Maximum cell lysis rates in the presence of gramicidin S were observed at concentrations of 3–5 μM; ivermectin enhanced lysis across the entire range of concentrations used, 1–15 μM (Fig. 4, Table 1). Dalargin and selank individually in the absence of LTF, lyse *E. coli* cells at concentrations of 0.1–0.6 μM and 0.1–1.4 μM, respectively. However, no lysis was observed when either of them was used in combination with LTF (Fig. 4, Table 1). This can presumably be explained by the adsorption of these oligopeptides on the protein surface.

Evaluating how surfactants modulate LTF bacteriolytic activity is of particular importance. Recent studies have shown that surfactant Tween-21 acts as a lysozyme effector, increasing its activity on *Lactobacillus plantarum* cells [25]. The chosen concentrations range of Tween-21 does not exceed

critical micelle concentration (CMC) [46]. We observed 3-fold to 3.2-fold increase in LTF activity on *E. coli* cells with the addition of Tween-21 at concentrations of 0.1–0.2 μM (Fig. 2D).

Lysozyme (bacteriolytic enzyme, muramidase, EC 3.2.1.17) through its active site exhibits specific activity by cleaving β-1,4-glycosidic bonds in the peptidoglycan murein of the bacterial cell wall. Since the outer (relative to the murein layer) membrane of Gram-negative bacteria is negatively charged, we hypothesized that a synergistic effect could be achieved by using a highly cationic protein such as lactoferrin in combination with lysozyme. LTF, interacting with the phosphate groups and lipopolysaccharides of bacterial membranes, would disrupt their integrity and allow lysozyme greater access to the peptidoglycan layer compared to the treatment of bacterial cells with lysozyme alone.

Figure 4 demonstrates the combined bacteriolytic action of lysozyme and lactoferrin, wherein the rate of *E. coli* lysis by lysozyme in the presence of LTF is 2.3-fold higher than the sum of the lysis rates by lysozyme and lactoferrin alone. Thus, the combined use of these two proteins is not the sum of the effects of each protein individually, but rather demonstrates a synergistic effect. This result allows us to propose a combination of two proteins – lysozyme and lactoferrin – as an effective treatment against bacterial infection. This combination, like the amino acid supplementation described above, is safe for human use, as all these compounds are normally present in the human body.

Conclusions

The study demonstrated that human lactoferrin can lyse intact *E. coli* cells and is activated in the presence of various low-molecular-weight effectors (charged amino acids, glycine, surfactant Tween-21, linear and cyclic oligopeptides), as well as in combination with lysozyme, demonstrating the synergism of their action. The mechanism of bacteriolytic action of LTF can presumably be either cationic, enzymatic, or a combination of both.

The amino acids arginine, glutamate, histidine, and glycine at concentrations of 2–7 mM increase LTF activity 2.2- to 4.5-fold. Crucially, these amino acids are non-toxic to cells, because they are normally occur in their free form within the human body and are safe for use in various medicinal compositions and dietary supplements [24]. We emphasize that studying the effect of standard ("canonical") amino acids as additives capable of influencing the effectiveness of bacteriolytic agents is a highly relevant scientific and practical task. Tween-21 at concentrations of 0.1–0.2 μM increases it 3.2-fold. The oligopeptides bacitracin, imunofan, and semax at concentrations of 1–5 μM are capable of increasing LTF activity 2- to 12-fold. Gramicidin S and ivermectin have no effect on LTF, but at concentrations of 5–15 μM, they lyse *E. coli* cells. Dalargin and selank individually at concentrations of 0.05–0.15 μM lyse *E. coli* cells, but no enhancement of lysis is observed when combined with LTF; it can be explained by the adsorption of these peptides on the LTF surface.

The experimental results demonstrate that native proteins and enzymes, as well as their various combinations designed to suppress bacterial infections, can either serve as alternatives to conventional antibiotics or enhance their efficacy via potential synergistic effects. Our results might contribute to the

development of novel approaches in drug design and improve our understanding of the fundamental mechanisms underlying the bacteriolytic (both enzymatic and non-enzymatic) activity of native macromolecules.

In summary, our findings highlight the therapeutic potential of lactoferrin and various lactoferrin-based combinations for treating bacterial infections, including those caused by antibiotic-resistant strains.

Declarations

Ethics declarations

This article does not contain any description of studies involving human participants or use of animals as research subjects.

Competing interests

The authors declare no conflict of interest.

Author Contribution

D.A.K. and P.A.L. conceptualized the study, wrote the original draft, conducted formal data analysis, interpreted the data, and discussed the results. D.A.K. performed the experiments. Both authors reviewed and edited the final manuscript.

Acknowledgement

This study was supported by the grant No. 25-26-00144 from the Russian Science Foundation (https://rscf.ru/project/25-26-00144/) "Activation of antibacterial bacteriolytic milk proteins with low-molecular-weight additives."

Data Availability

All data supporting the findings of this study are available within the article. Source data for this study are deposited in the Zenodo repository under DOI: 10.5281/zenodo.21293739

References

1. World Health Organization (WHO). Global antibiotic resistance surveillance report, WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) (2025) ISBN 978-92-4-011634-4. https://www.who.int/publications/i/item/9789240116337 (accessed 2026-07-01)

2. Li X (2025) Molecular Mechanisms of Antibiotic Resistance in Bacteria and Alternative Treatment Strategies: From Evolution of Resistance to Precision Antimicrobial Therapy. Theor Nat Sci 131:53–59. https://doi.org/10.54254/2753-8818/2025.SH26350
3. Miftode IL, Radu VD, Jigoranu RA, Leca DA, Prepeliuc CS, Pasare MA, Miftode RS, Grigoriu MG, Parângă TG, Miftode EG (2026) Novel Insights into Carbapenem Resistance: Mechanisms, Diagnostics, and Future Directions. Antibiotics 15(3):270. https://doi.org/10.3390/antibiotics15030270
4. Sartori M, Toppo S, Lavezzo E (2026) Molecular resistance mechanisms to newly approved antibiotics (2017–2025) in WHO priority pathogens. Front Microbiol 16:1719798. https://doi.org/10.3389/fmicb.2025.1719798
5. Xie Y, Lu H, Liu Y, Hu G, Lian S, Liu J, Pang S, Zhu G, Ding X (2025) Unveiling the Mechanisms of Bacterial Resistance and Countermeasures. Pathogens 14:1085. https://doi.org/10.3390/pathogens14111085
6. Kim TJ (2026) Bacterial Persister Cells as Evolutionary Catalysts of Antibiotic Resistance: Mechanisms, Clinical Implications, and Therapeutic Strategies. Antibiotics 15:526. https://doi.org/10.3390/antibiotics15060526
7. Tayeb SM, Alharbi JA, Alattas BB, Alotaibi DM, Althibaiti NM, Alharbi JF, SafirAldeen AE, Alqurashi IR, Wali SM (2025) Promising Future of Novel Beta-Lactam Antibiotics Against Bacterial Resistance. Drug Des Devel Ther 19:9185–9197. https://doi.org/10.2147/dddt.s551862
8. Krawczyk SJ, Leśniczak-Staszak M, Gowin E, Szaflarski W (2024) Mechanistic Insights into Clinically Relevant Ribosome-Targeting Antibiotics. Biomolecules 14:1263. https://doi.org/10.3390/biom14101263
9. El-sagheir AMK, Wenzel M, Yli-Kauhaluoma J (2025) Fluoroquinolones as versatile scaffolds: Potential for targeting classical and novel mechanisms to combat antibacterial resistance. Eur J Pharm Sci 214:107247. https://doi.org/10.1016/j.ejps.2025.107247
10. Kumar R (2025) Expanding horizon of antimicrobial peptides: mechanistic complexity and biomedical potential. Explor Drug Sci 3:1008135. https://doi.org/10.37349/eds.2025.1008135
11. Rizzi M, Manzoni P, Germano C, Quevedo MF, Sainaghi PP (2025) Lactoferrin, a Natural Protein with Multiple Functions in Health and Disease. Nutrients 17:3403. https://doi.org/10.3390/nu17213403
12. Sienkiewicz M, Jaśkiewicz A, Tarasiuk A, Fichna J (2022) Lactoferrin: an overview of its main functions, immunomodulatory and antimicrobial role, and clinical significance. Crit Rev Food Sci Nutr 62:6016–6033. https://doi.org/10.1080/10408398.2021.1895063
13. Hong R, Xie A, Jiang C, Guo Y, Zhang Y, Chen J, Shen X, Li M, Yue X (2024) A review of the biological activities of lactoferrin: mechanisms and potential applications. Food Funct 15:8182–8199. https://doi.org/10.1039/d4fo02083a
14. Bolesławska I, Bolesławska-Król N, Jakubowski K, Przysławski J, Drzymała-Czyż S (2025) Lactoferrin – A Regulator of Iron Homeostasis and Its Implications in Cancer. Molecules 30:1507. https://doi.org/10.3390/molecules30071507

15. Kim JW, Lee JS, Choi YJ, Kim C (2025) The Multifaceted Functions of Lactoferrin in Antimicrobial Defense and Inflammation. Biomolecules 15:1174. https://doi.org/10.3390/biom15081174
16. Shafqat A, Li M, Zakirullah, Liu F, Tong Y, Fan J, Fan H (2025) A comprehensive review of research advances in the study of lactoferrin to treat viral infections. Life Sci 361:123340. https://doi.org/10.1016/j.lfs.2024.123340
17. Yami HA, Tahmoorespur M, Javadmanesh A, Tazarghi A, Sekhavati MH (2023) The immunomodulatory effects of lactoferrin and its derived peptides on NF-κB signaling pathway: A systematic review and meta-analysis. Immun Inflamm Dis 11:e972. https://doi.org/10.1002/iid3.972
18. Rascón-Cruz Q, Espinoza-Sánchez EA, Siqueiros-Cendón TS, Nakamura-Bencomo SI, Arévalo-Gallegos S, Iglesias-Figueroa BF (2021) Lactoferrin: A Glycoprotein Involved in Immunomodulation, Anticancer, and Antimicrobial Processes. Molecules 26:205. https://doi.org/10.3390/molecules26010205
19. Bukowska-Ośko IA, Sulejczak D, Kaczyńska K, Kleczkowska P, Kramkowski K, Popiel M, Wietrak E, Kowalczyk P (2022) Lactoferrin as a Human Genome Guardian - An Overall Point of View. Int J Mol Sci 23:5248. https://doi.org/10.3390/nu17213403
20. Ongena R, Dierick M, Vanrompay D, Cox E, Devriendt B (2024) Lactoferrin impairs pathogen virulence through its proteolytic activity. Front Vet Sci 11:1428156. https://doi.org/10.3389/fvets.2024.1428156
21. Angulo-Zamudio UA, Vidal JE, Nazmi K, Bolscher JGM, Leon-Sicairos C, Antezana BS, Canizalez-Roman A, León-Sicairos N (2019) Lactoferrin Disaggregates Pneumococcal Biofilms and Inhibits Acquisition of Resistance Through Its DNase Activity. Front Microbiol 10:2386. https://doi.org/10.3389/fmicb.2019.02386
22. Aguila A, Herrera AG, Morrison D, Cosgrove B, Perojo A, Montesinos I, Pérez J, Sierra G, Gemmell CG, Brock JH (2001) Bacteriostatic activity of human lactoferrin against Staphylococcus aureus is a function of its iron-binding properties and is not influenced by antibiotic resistance. FEMS Immunol Med Microbiol 31:145–152. https://doi.org/10.1111/j.1574-695x.2001.tb00511.x
23. Klimov DA, Levashov PA (2025) Structural Features and Properties of Transferrins: A Review. Appl Biochem Microbiol 61:1003–1033. http://dx.doi.org/10.1134/S0003683825600903
24. Rastriga NV, Klimov DA, Gasanova DA, Levashov PA (2023) Comparison of the individual and combined actions of charged amino acids and glycine on the lysis of *Esherichia coli* cells by human and chicken lysozyme. Process Biochem 125:190–197. https://doi.org/10.1016/j.procbio.2022.12.024
25. Lu WJ, Smirnov SA, Levashov PA (2021) General characteristics of the influence of surfactants on the bacteriolytic activity of lysozyme based on the example of enzymatic lysis of *Lactobacillus plantarum* cells in the presence of Tween 21 and SDS. Biochem Biophys Res Commun 575:73–77. https://doi.org/10.1016/j.bbrc.2021.08.060
26. Levashov PA, Matolygina DA, Ovchinnikova ED, Atroshenko DL, Savin SS, Belogurova NG, Smirnov SA, Tishkov VI, Levashov AV (2017) Bacteriolytic activity of human interleukin-2, chicken egg

lysozyme in the presence of potential effectors. Acta Naturae 9:82–87. http://dx.doi.org/10.32607/20758251-2017-9-2-82-87

27. Liashev AY, Mal GS, Solin AV, Balanina MA (2024) The effect of dalargin on growth factors content in experimental ulcerative colitis. Res Results Pharmacol 10:67–73. https://doi.org/10.18413/rrpharmacology.10.430
28. Radchenko AI, Kuzubova EV, Apostol AA, Mitkevich VA, Andreeva LA, Limborskaya SA, Stepenko YV, Shmigerova VS, Solin AV, Korokin MV, Pokrovskii MV, Myasoedov NF, Makarov AA (2025) The potential of the peptide drug Semax and Its derivative for correcting pathological impairments in the animal model of Alzheimer's disease. Acta Naturae 17:110–120. https://doi.org/10.32607/actanaturae.27808
29. Vyunova TV, Andreeva L, Shevchenko K, Myasoedov N (2018) Peptide-based Anxiolytics: The Molecular Aspects of Heptapeptide Selank Biological Activity. Protein Pept Lett 25:914–923. https://doi.org/10.2174/0929866525666180925144642
30. Sawicka J, Dzierżyńska M, Wardowska A, Deptuła M, Rogujski P, Sosnowski P, Filipowicz N, Mieczkowska A, Sass P, Pawlik A, Hać A, Schumacher A, Gucwa M, Karska N, Kamińska J, Płatek R, Mazuryk J, Zieliński J, Kondej K, Młynarz P, Mucha P, Skowron P, Janus Ł, Herman-Antosiewicz A, Sachadyn P, Czupryn A, Piotrowski A, Pikuła M, Rodziewicz-Motowidło S (2020) Imunofan—RDKVYR Peptide—Stimulates Skin Cell Proliferation and Promotes Tissue Repair. Molecules 25:2884. https://doi.org/10.3390/molecules25122884
31. Ashrafuzzaman M, Andersen OS, McElhaney RN (2008) The Antimicrobial Peptide Gramicidin S Permeabilizes Phospholipid Bilayer Membranes Without Forming Discrete Ion Channels. Biochim Biophys Acta 1778:2814–2822. https://doi.org/10.1016/j.bbamem.2008.08.017
32. Buijs NP, Vlaming HC, Kotsogianni I, Arts M, Willemse J, Duan Y, Alexander FM, Cochrane SA, Schneider T, Martin NI (2024) A classic antibiotic reimagined: Rationally designed bacitracin variants exhibit potent activity against vancomycin-resistant pathogens. Proc. Natl. Acad. Sci. USA. 121:2315310121. https://doi.org/10.1073/pnas.2315310121
33. Crump A, Omura S (2011) Ivermectin, 'Wonder drug' from Japan: the human use perspective. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 87:13–28. https://doi.org/10.2183/pjab.87.13
34. Baker EN, Baker HM, Kidd RD (2002) Lactoferrin and transferrin: Functional variations on a common structural framework. Biochem Cell Biol 80:27–34. https://doi.org/10.1139/o01-153
35. Sindi AS, Stinson LF, Lai CT, Gridneva Z, Leghi GE, Netting MJ, Wlodek ME, Muhlhausler BS, Zhou X, Payne MS, Geddes DT (2025) Human milk lactoferrin and lysozyme concentrations vary in response to a dietary intervention. J Nutr Biochem 135:109760. https://doi.org/10.1016/j.jnutbio.2024.109760
36. Osuna-Gómez R, Mulet M, Barril S, Cantó E, Millan-Billi P, Pardessus A, de la Rosa-Carrillo D, Castillo D, Vidal S (2024) Levels of Lysozyme and SLPI in Bronchoalveolar Lavage: Exploring Their Role in Interstitial Lung Disease. Int J Mol Sci 25:4297. https://doi.org/10.3390/ijms25084297

37. Hanstock HG, Edwards JP, Walsh NP (2019) Tear Lactoferrin and Lysozyme as Clinically Relevant Biomarkers of Mucosal Immune Competence. Front Immunol 10:1178. https://doi.org/10.3389/fimmu.2019.01178
38. Ashraf MF, Zubair D, Bashir MN, Alagawany M, Ahmed S, Shah QA, Buzdar JA, Arain MA (2024) Nutraceutical and Health-Promoting Potential of Lactoferrin, an Iron-Binding Protein in Human and Animal: Current Knowledge. Biol Trace Elem Res 202:56–72. https://doi.org/10.1007/s12011-023-03658-4
39. Abdallah EM, Alhudhaibi AM, Dahab M, Al Noman A, Sharma PD, Taha TH, Nawaz M (2026) The WHO priority list of antibiotic-resistant bacteria: challenges and opportunities for next-generation antimicrobial developmen. Front Pharmacol 17:1699987. https://doi.org/10.3389/fphar.2026.1699987
40. Overberger CG, Kawakami Y (1978) Esterolytic activity of imidazole-containing polymers. Hydrophobic influences in copoly[1-alkyl-4- or 5-vinylimidazole/4(5)-vinylimidazole]-catalyzed hydrolysis of 3-nitro-4-acyloxybenzoic acids. J Polym Sci Polym Chem Ed 16:1249–1263. https://doi.org/10.1002/pol.1978.170160608
41. Kunitake T, Okahata Y (1976) Catalytic Hydrolysis by Synthetic Polymers. Adv Polym Sci 20:159–221. https://doi.org/10.1007/BFb0023971
42. Baker EN, Baker HM, Kidd RD (2009) A structural framework for understanding the multifunctional character of lactoferrin. Biochimie 91:3–10. https://doi.org/10.1016/j.biochi.2008.05.006
43. Harata K, Muraki M, Hayashi Y, Jigami Y (1992) X-ray structure of Glu 53 human lysozyme. Protein Sci 1:1447–1453. https://doi.org/10.1002/pro.5560011106
44. Rouchon CN, Harris J, Zubair-Nizami Z, Weinstein AJ, Roky M, Frank KL (2022) The Cationic Antimicrobial Peptide Activity of Lysozyme Reduces Viable Enterococcus faecalis Cells in Biofilms. Antimicrob Agents Chemother 66:02339–02321. https://doi.org/10.1128/aac.02339-21
45. Scanlon TC, Teneback CC, Gill A, Bement JL, Weiner JA, Lamppa JW, Leclair LW, Griswold KE (2010) Enhanced antimicrobial activity of engineered human lysozyme. ACS Chem Biol 5:809–818. https://doi.org/10.1021/cb1001119
46. Di Marzio L, Marianecci C, Petrone M, Rinaldi F, Carafa M (2011) Novel pH-sensitive non-ionic surfactant vesicles: comparison between Tween 21 and Tween 20. Colloids Surf B Biointerfaces 82:18–24. https://doi.org/10.1016/j.colsurfb.2010.08.004

Figures

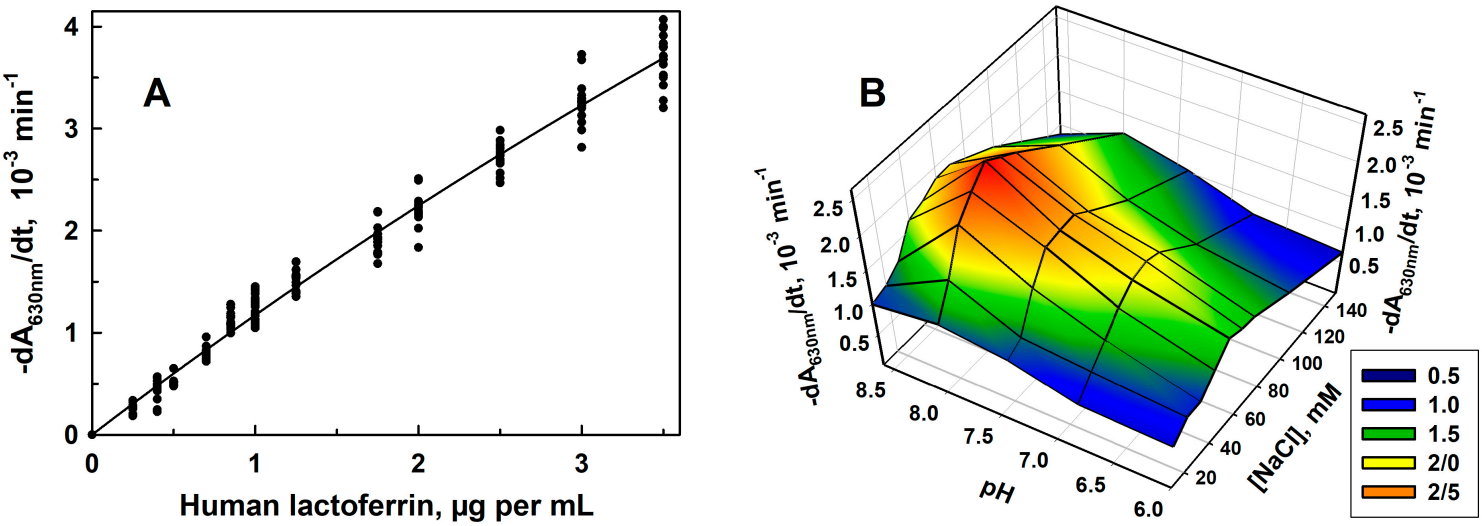


Figure 1

A: Lysis of *E. coli* cells by human LTF at a fixed LTF concentration of 2.5 μg/mL. **B:** Dependence of the rate of E. coli cell lysis on pH and NaCl concentration at the fixed value of [LTF] = 2.5 μg/mL.

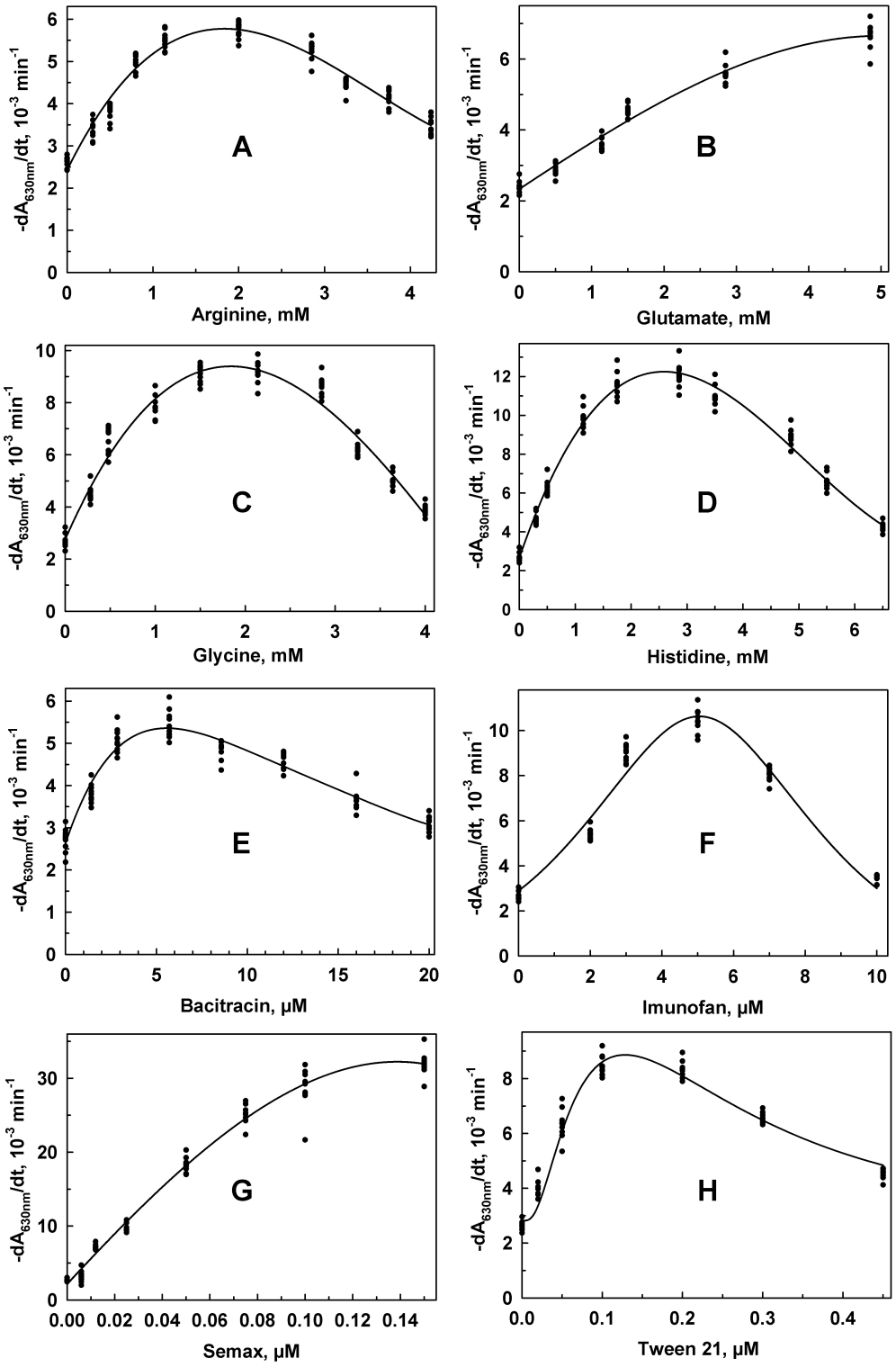


Figure 2

Lysis of *E. coli* cells by human LTF in the presence of free amino acids, bacitracin, imunofan, semax (MEHFPGP) and Tween-21 at the fixed value of [LTF] = 2.5 μg/mL. These additives do not, by themselves, cause cell lysis.

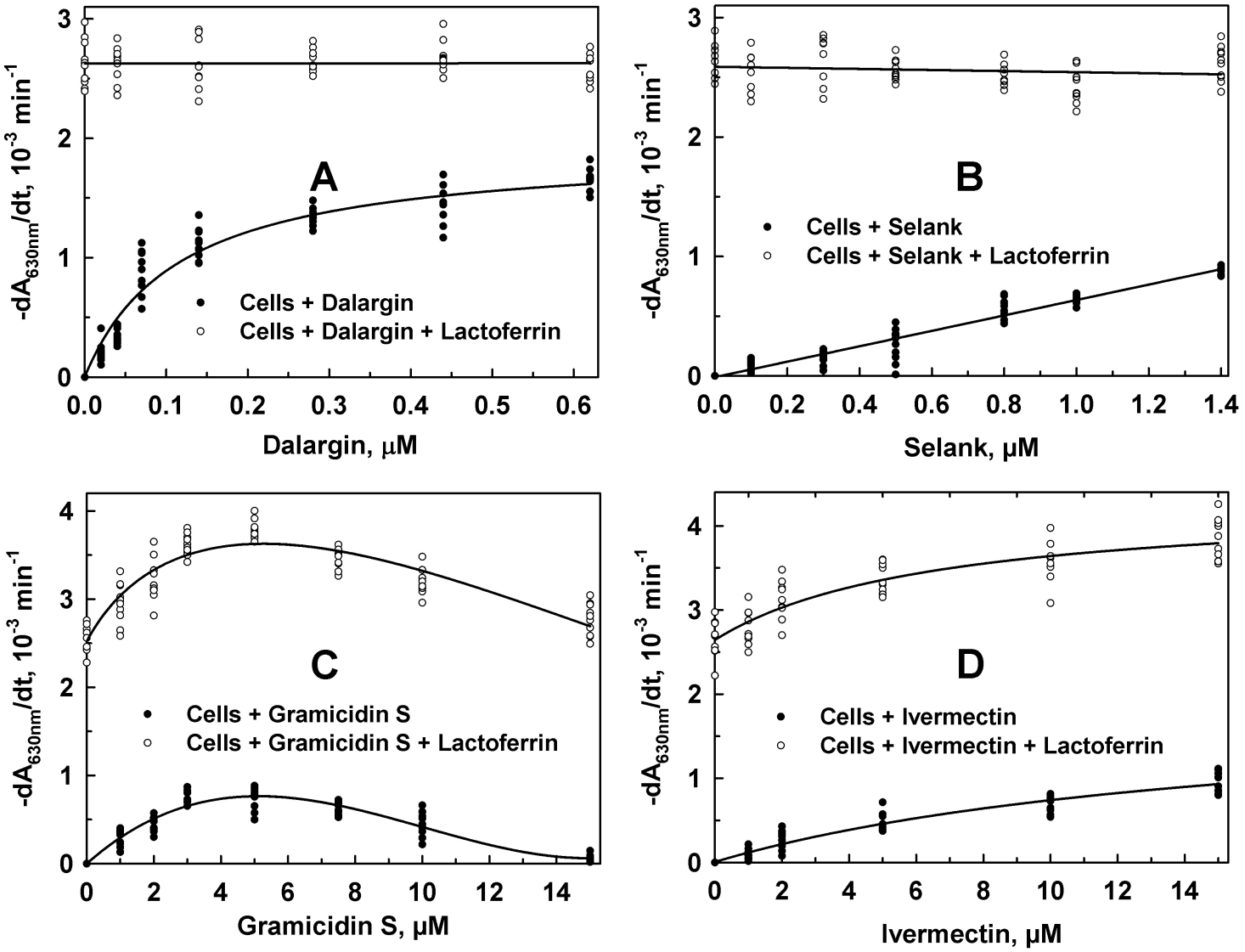


Figure 3

Lysis of *E. coli* cells by human LTF in the presence of gramicidin S, dalargin, ivermectin and selankat the fixed value of [LTF] = 2.5 μg/mL. These additives also induce cell lysis on their own, in the absence of lactoferrin.

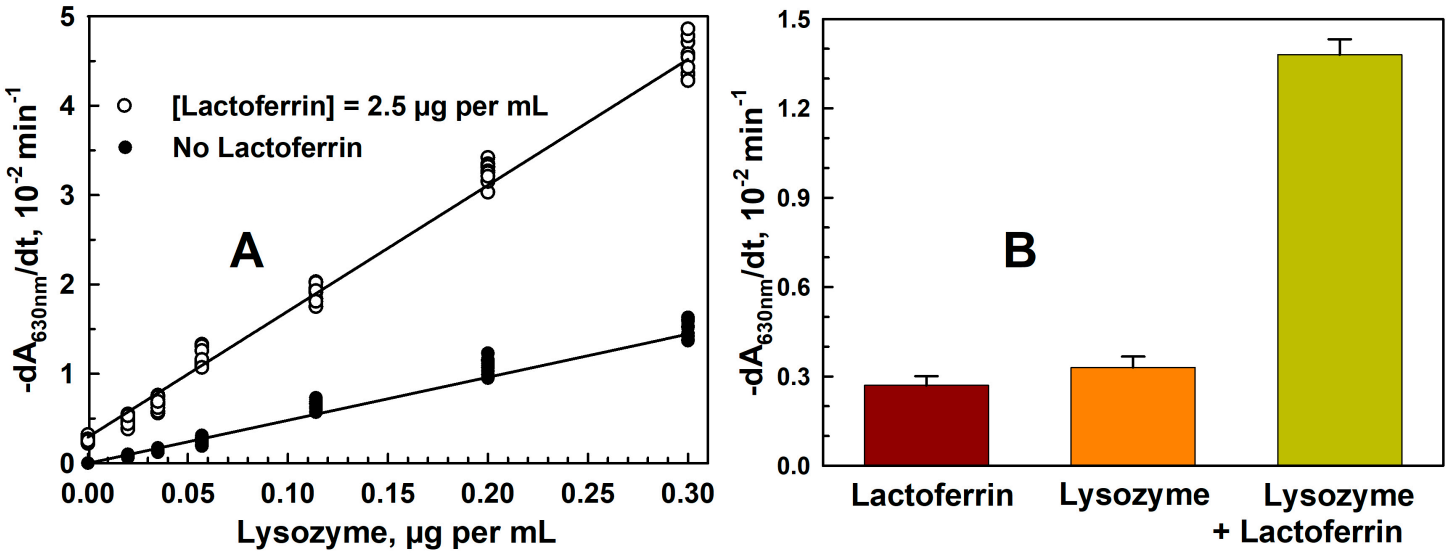


Figure 4

A. Kinetics of *E. coli* lysis by human lysozyme with and without human lactoferrin. **B.** Individual and combined bacteriolytic action of lysozyme (LZM) and lactoferrin on *E. coli* cells at the fixed values of [LZM] = 0.075 µg/mL, [LTF] = 2.5 µg/mL; in combination ([LZM] + [LTF]) the same concentrations were used. The error was calculated using the Student's t-distribution for a 0.95 confidence interval.

Supplementary Files

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