Volume 7, Issue 3, 2026

Trends in Natural Products Research

Influence of Glycerogelatin on the Antibacterial Activity of *Limosilactobacillus reuteri* CF48-3A Against *Escherichia coli.*

Enosakhare Oloton* and Amomatorione Excellent Yaluwu

Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Abstract

Escherichia coli is one of the most frequently encountered bacteria in aerobic vaginitis. This study evaluated the influence of glycerogelatin base on the antibacterial activity of *Limosilactobacillus reuteri* CF48-3A against *Escherichia coli* ATCC 25922. Both strains were characterized using phenotypic and biochemical methods. Antibacterial activities were assessed using the agar overlay method, while co-cultivation assessed comparative *E. coli* growth inhibitory patterns in combination with *L. reuteri* CF48-3A and glycerogelatin base. Statistical significance was determined using two-factor ANOVA. Characterization confirmed the identity of both organisms. The agar overlay method demonstrated only bacteriostatic activity (IZD = 30.5 ± 0.44). Co-cultivation reduced *E. coli* growth to 0.38 $\log_{10}$ CFU/mL/h (21.4%) in the presence of *L. reuteri* CF48-3A compared with control, indicating antagonism. An addition of glycerogelatin to the system further reduced to 0.18 $\log_{10}$ CFU/mL/h (56.1%). This reduction was enhanced with the presence of glycerogelatin base. Glycerogelatin produced a highly significant antibacterial activity (Two way ANOVA), with Turkey's post hoc analysis showing significant differences ($P < 0.05$) compared to other groups. *L. reuteri* CF48-3A is a promising probiotic candidate for inhibiting *E. coli* ATCC 25922, while, glycerogelatin base is an effective base for optimizing its antibacterial activity.

Keyword: *Escherichia coli* ATCC 25922, Glycerogelatin, *Limosilactobacillus reuteri* C3A, Probiotics antagonism, Probiotic pessaries.

*Corresponding author:
enosakhare.oloton@uniben.edu
+2348036541222
https://doi.org/10.61594/tnpr.v7i3.2026.177
Page No.: 307-313
Volume: Volume 7 Issue 3, 2026
Trends in Natural Products Research

Received 26/5/26, Revised 30/5/26, Revised 5/6/26, Published Online 7/7/26

Introduction

Escherichia coli (*E. coli*) are rod-shaped, Gram-negative coliform bacteria that are widely distributed in nature. They are a large and diverse group of bacteria, and they can cause infections to both humans and animals. Most *E. coli* strains are harmless and part of the human microbiota. However, some strains are pathogenic and can cause severe human illness (Altaie *et al.,* 2025). Pathogenic strains of *E. coli* can be distinguished from the normal microbiota by their virulence factors such as exotoxins, are responsible for significant diseases in both humans and animals (Spickler, 2016). *E. coli* is one of the commonly encountered bacteria in aerobic vaginitis (AV) (Zhang *et al.,* 2020). This is a known form of vaginal dysbiosis presenting with an epithelial disruption, purulent discharge and significant inflammation (Donders *et al.,*2017). Attention has shifted toward the use of probiotics as natural antagonists of pathogenic bacteria. *Limoslactobacillus reuteri* CF48-3A (formerly known as *Lactobacillus reuteri* CF48-3A) has been documented to produce reuterin a potent anti-pathogenic compound produced by *L. reuteri* and capable of inhibiting a wide spectrum of microorganisms including gram-positive bacteria, Gram negative bacteria, fungi, and protozoa (Jones and Versalovic, 2009). This is indicative of a probiotic candidate suitable for possible adjunct management of *Escherichia coli* induced aerobic vaginitis. Research had shown the stability profiling of probiotic pessaries in glycerogelatin base (Ebenebe *et al.,*2021). There is limited published evidence regarding the impact of the base on the probiotic organism used in the formulation. In the preparation of probiotic pessaries, there is need to select appropriate bases that will not negatively interfere with release or absorbance of the active pharmaceutical ingredient (Kumar *et al.,* 2023). Glycerogelatin is one of the possible bases that can be used for preparation of *Lactobacillus* suppositories (Melnyk *et al.,* 2020). Therefore, there is need to preliminarily identify the most suitable base compactible with the release and antipathogenic activity of this preparation.

Despite several emerging trends in probiotic–pathogen antagonistic interactions, few studies (Li *et al.,* 2026) have addressed the influence of glycerogelatin base in the inhibitory activity of *Limosilactobacillus reuteri* CF48-3A against *Escherichia coli* in co-culture systems. Understanding these preliminary interactions may provide valuable insight into choosing the most appropriate base for future preparation and experimentations of probiotic pessary. Therefore, this study aimed to evaluate the Influence of glycerogelatin on the antibacterial activity of *Limosilactobacillus reuteri* CF48-3A against *Escherichia coli.*

Materials and Methods

Materials

The Biological materials used were *Limoslactobacillus reuteri* CF48-3A, as the probiotics strain, and Escherichia *coli* ATCC 25922, as the indicator organism for antibacterial evaluation. Other chemical and reagents used are Mueller Hinton agar and Mueller Hinton broth (Titan Biotech Ltd, India), eosin methylene blue agar (Ready MED, India), De Man-Rogosa-Sharpe (MRS)Agar and broth (Titan Biotech, India), Gelatin (Moly chem, India), Hydrogen peroxide (CDH, India), Sucrose, Lactose, Maltose, D-glucose, D-mannitol, L-rhamnose, D-xylose, (Laboratory Burgoyne reagent, India), D-fructose (Trust chemical Laboratory, China), crystal violet (Scharlau, Spain), oil immersion (Sisco Research Laboratory, India), ethanol (JHD, China), Safranine (Qualikems, India), sodium dihydrogen phosphate dihydrate, potassium Dihydrogen Phosphate, sodium Chloride (GH TECH, China) and Lugo's Iodine (Bema scientific and chemical Ltd, Nigeria) and glycerol. The equipment used in this study included a Microscope (Biobase, China), incubator (Dai Han Scientific Ltd., South Korea), autoclave. (Prestige series, UK) Refrigerator (Haier Thermocool, Nigeria), Micropipettes 1000 and 200 µL capacity (Microlit, India), Weighing balance (Ohaus, USA), PH meter (Hanna instruments, Italy) and Water distiller.SZ-96 Biochemistry instrument factory (China)

Methods

Bacterial Phenotypic and Biochemical Characterization

The *L. reuteri* CF48-3A and *Escherichia coli* ATCC 25922 were characterized using phenotypic and biochemical methods according to Cheesbrough (2010). Gram reaction was determined by Gram staining, while catalase activity was determined using 3% hydrogen peroxide. Carbohydrate fermentations were conducted using sucrose, lactose, maltose, D-glucose, D-fructose, D-mannitol, L-rhamnose, and D-xylose. Biochemical characterization included methyl red, Voges–Proskauer, citrate utilization, and urease tests for *Escherichia coli* ATCC 25922. The outcomes were authenticated based on documented literature.

Preliminary antibacterial studies

The antibacterial activity of *L. reuteri* CF48-3A against *Escherichia coli* ATCC 25922 was assessed using the Agar overlay method (Abhyankar and Kapadnis 2021) with specific modifications. *L. reuteri*

CF48-3A was cultured in de Man, Rogosa, and Sharpe (MRS) broth at 37℃ for 48 h. Fifty microliters (50 µL) of the culture was spotted onto the surface of 20 mL solidified sterile MRS agar and incubated at 37 ℃ for 48 h to allow spot-confluent growth in microaerophilic conditions. The agar plates were subsequently layered with 10 mL of Muller-Hinton (MH) agar pre-inoculated with 100 µL of 18 h culture of *E. coli* ATCC 25922. The MH-layered plates were allowed to set and incubated for 18 h at 37 ℃. Agar scrapings from the inhibition zones were aseptically subcultured in nutrient broth. After incubation at 37 °C for 24 h, the bacteriostatic and bactericidal activities were determined (Balouiri *et al.,*2026)

Probiotic-pathogen cocultivation assay

The growth inhibition rates of *E. coli* ATCC 25922 were determined using the cocultivation assay as previously documented (Denkova *et al.,* 2013) with specific modifications. The modifications included addition of suppository bases and prolongation of the time of assessment. *E. coli* ATCC 25922 (3.0 X 10^4 CFU/mL) was cultured in joint combinations with 2.25 X 10^4 CFU/mL of *L. reuteri* CF48-3A, glycerogelatin in MRS, MH broth, sterile distilled water (SDW). The following (Table 1) were prepared and 100 µL were collected at 4-hour intervals over a 24 h period.

Table 1: Probiotic-pathogen-base system

Co culture combinations	MRS (mL)	MH (mL)	SDW (mL)	Glycerogelatin (mL)
E. coli (control)	1.5	1.5	6	-
E. coli + *L. reuteri* CF48-3A	1.5	1.5	6	-
E. coli+ *L. reuteri* CF48-3A + Glycerogelatin	1.5	1.5	3	3

Serial tenfold dilutions of all samples were made in phosphate buffer saline (PBS) and plated in Eosin Methylene Blue agar for enumeration of the *E. coli* in duplicate. The growth inhibition (%) were calculated with the growth rate (slope) of the combinations compared with control.

Statistical Analysis

Data was analyzed using two-way analysis of variance (ANOVA) with SPSS version 27.0 (IBM Corp., Armonk, NY, USA), followed by Turkey's HSD post hoc test for multiple comparisons. Differences between groups were considered statistically significant at $P < 0.05$.

Results

Bacterial and Phenotypic and biochemical characterization

L. reuteri CF48-3A was identified as a Gram-positive bacillus and catalase-negative (Table 2). This strain fermented sucrose, lactose, maltose, D-fructose, and D-glucose, while showing no fermentation of D-mannitol, L-rhamnose, and D-xylose. While *E. coli* ATCC 25922 was identified as a Gram-negative bacillus, catalase positive organism, it also demonstrated the ability to ferment lactose, glucose, maltose, fructose, mannitol, rhamnose, and xylose. The organism was methyl red positive, and negative for sucrose, Voges–Proskauer, citrate, and urease tests.

Table 2: Phenotypic and Biochemical Characteristics

Test	Specific interactions	*L. reuteri* CF48-3A	*E. coli ATCC 25922*
Gram staining	Gram reaction	GPB	GNB
Catalase test	Hydrogen peroxide	-	+
Carbohydrate fermentation	Sucrose	+	-
	Lactose	+	+
	Maltose	+	+
	D-Fructose	+	+
	D-Glucose	+	+
	D-Mannitol	-	+
	L-Rhamnose	-	+
	D-Xylose	-	+
Biochemical Test	Methyl Red	NT	+
	Voges-Proskauer	NT	-
	Citrate	NT	-
	Urease	NT	-

GPB: Gram -positive bacilli, GNB: Gram-negative bacilli, ND: Not tested.
+: Positive, -: Negative

Preliminary antibacterial studies.

L. *reuteri* CF48-3A demonstrated a strong antibacterial activity against *E. coli* ATCC 25922, with inhibition zone of 30.5 ± 0.44 mm. However, growth was observed during the bactericidal evaluation.

Probiotic-pathogen cocultivation assay

The untreated *E. coli* showed unrestricted bacterial growth with a regression equation of $y = 5.71 + 0.41x$ with a strong positive correlation ($r = 0.902$), In combination with *L. reuteri* CF48-3A alone reduced bacterial growth ($y = 3.24 + 0.38x$; $r = 0.933$) which corresponds to 21.4% inhibition in comparison with control (Figure 1). A greater antagonistic effect was observed in the *L. reuteri* and glycerogelatin addition which showed regression equation and Pearson correlation of $y = 2.92 + 0.18$; $r = 0.667$ and 56.1% inhibition of *E. coli.* The two way ANOVA exhibited a significant effect of both treatment (Log CFU/mL) and time (24 h) on the bacterial count ($P < 0.001$). Further analysis with Turkey's HSD post hoc demonstrated significant differences among all treatment groups ($P < 0.05$): *E. coli* combined with *L. reuteri* and Glycerogelatin [a] *E. coli* [b] while *E. coli* combined with *L. reuteri* [c] (Figure 1).

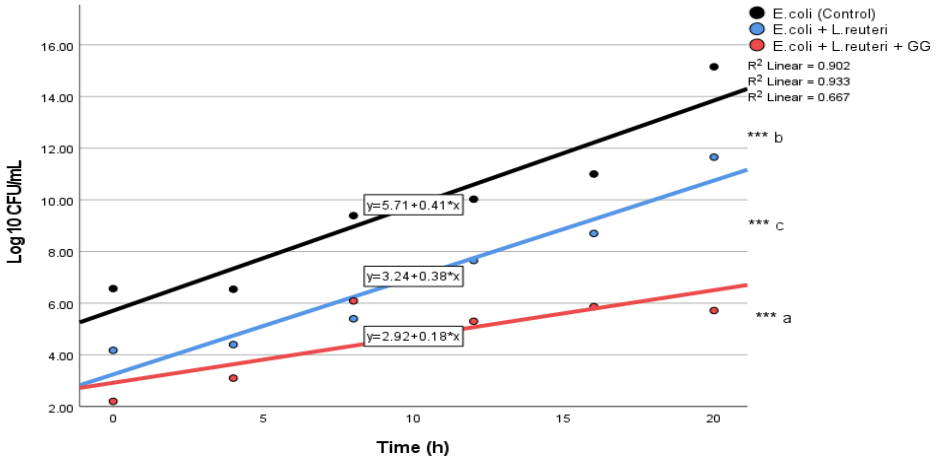

Figure 1: Time dependent inhibition pattern of *E. coli* (control), *E. coli* (21.4%) with *L. reuteri* CF48-3A and *E. coli* (56.1%) with glycerogelatin. A Significant effect of time and treatments were observed by Two way ANOVA (*** $P < 0.001$). Different letters (a-c) signify significant differences among the treatment groups according to Turkey's HSD post hoc analysis ($P < 0.05$).

Discussion

The strain *L. reuteri* CF48-3A displayed the classical traits of lactic acid bacteria, being a Gram-positive rod and catalase-negative. This specific pattern of carbohydrate assimilation is consistent with previously documented biochemical characteristics of *L. reuteri* (Holzapfel and Wood, 2014). These phenotypic properties provide strong evidence supporting the accurate identification and authentication of this *Lactobacillus* strain, while *Escherichia coli* ATCC 25922 characteristics are consistent with the established biochemical profile of *E. coli* (Holt *et al.,* 1994; MacFaddin, 2000). These phenotypic traits collectively validate the authentication of both strains employed in this present study. The reproducibility of the Gram reaction and enzyme activity to carbohydrate fermentation patterns provides a foundation for their use as authenticated model organisms.

The *L. reuteri* CF48-3A displayed antagonism toward *Escherichia coli* ATCC 25922. According to IZD classification, this activity is considered strong, though subsequent bactericidal assays confirmed persistence of growth, thereby defining the effect as bacteriostatic impact rather than bactericidal (Shokryazdan *et al.,* 2014). Similar strains have been shown to reduce *E. coli* population in an *in vitro* fermentation model (Cleusix *et al.,* 2008). The untreated *E. coli* control showed a clear increase in survival over time, with a growth rate of 0.41$\log_{10}$ CFU/mL per unit time and a strong positive Pearson correlation. This indicates that, in the absence of inhibition, *E. coli* growth was strongly time dependent. This establishes a valid baseline for assessing inhibitory effects. Co-cultivation with *L. reuteri* substantially reduced the *E. coli* growth rate from 0.41 to 0.38 $\log_{10}$ CFU/mL, representing to an estimated 21.4 % growth inhibition. The inhibitory mechanism may be linked to the secretion of reuterin and other diffusible metabolites known to be produced by *L. reuteri* CF48-3A (Jones and Versalovic, 2009). This metabolite breaks down glycerol to hydroxypropionaldehyde (3-HPA) (Talarico and Dobrogosz,1989). This step is catalyzed by glycerol dehydratase and assisted by coenzyme B12. 3-HPA is further converted into acrolein (Chen *et al.,* 2013), which specifically exerts cytotoxic effects which specifically exerts cytotoxic effects and inhibits the

growth of some gram-negative bacilli (Luo 2023). Collectively, these results buttress the functional probiotic potential of *L. reuteri* CF48-3A in controlling *E. coli* growth. The combination of *E. coli* with *L. reuteri* and glycerogelatin treatment demonstrated a greater reduced growth rate of 0.41 to 0.18 $\log_{10}$ CFU/mL, corresponding to a higher calculated 56.1% growth inhibition relative to the control, suggesting that the addition of glycerogelatin in the system significantly enhanced the antibacterial activity of *L. reuteri* CF48-3A against *E. coli.* Current literature (Li *et al.,* 2026) provides limited information regarding the influence of glycerogelatin on the antibacterial performance of probiotics against *E. coli*. Consequently, the observed differences may reflect enhanced diffusion with the incorporation of hydrophilic nature of glycerogelatin (Shende *et al.,*2023). The two-factor ANOVA without replication showed a statistically significant effect of both time and treatment combinations on *E. coli*. This confirms that changes in $\log_{10}$ CFU/mL were not random but were significantly influenced by both the 24 h time duration and base combinations. The distinct separation of the treatment group by Turkey's HSD analysis further buttresses significant differences among all the groups.

Conclusion

Limosilactobacillus reuteri CF48-3A exhibited significant antagonistic activity against *E. coli* ATCC 25922. Glycerogelatin base enhanced the antibacterial activity of the probiotic *L. reuteri* CF48-3A. These findings support the potential application of glycerogelatin-base in probiotic preparation for managing *E. coli*-associated infections.

Acknowledgment

The authors acknowledge the support of BEI Resources, National Institute of Allergy and Infectious Diseases, and National Institutes of Health. *The following reagent was obtained through BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Limosilactobacillus reuteri strain CF48-3A*

.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

References

Abhyankar PS, Kapadnis BP (2021). Antifungal activity of plant isolate of lactic acid bacteria against Fusarium sp. *Journal of Scientific Research* 65(5):116–128.

Altaie HAA, GdouraBen Amor M, Mohammed BA, Gdoura R (2025). Detection and characterization of *Escherichia coli* and *Escherichia coli* O157:H7 in human, animal and food samples from Kirkuk Province, Iraq. *Microbiological Research* 16, 20:2-21

Balouiri M, Sadiki M, Ibnsouda SK (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis* 6(2):71–79.

Cheesbrough M (2010). District Laboratory Practice in Tropical Countries, Part 2, Low Price Edition. Cambridge University Press, pp. 63-125.

Chen G, Chen J (2013). A novel cell modification method used in biotransformation of glycerol to 3-HPA by *Lactobacillus reuteri*. *Applied Microbiology and Biotechnology* 97 (10):4325–32.

Cleusix V, Lacroix C, Vollenweider S, Le Blay G (2008). Glycerol induces reuterin production and decreases *Escherichia coli* population in an in vitro model of colonic fermentation with immobilized human feces. *FEMS microbiology Ecology* 63(1):56–64.

Denkova R, Ilieva S, Nikolova D et al (2013) Antimicrobial activity of *Lactobacillus plantarum* X2 against pathogenic microorganisms. *Bulgarian Journal of Agricultural Science* 19(2):108–111.

Donders GGG, Bellen G, Grinceviciene S et al P (2017). Aerobic vaginitis: no longer a stranger. *Research in Microbiology* 168(9/10): 845–858.

Ebenebe IN, Uduma OE, Esimone CO, Anukam KC (2021). Development and evaluation of vaginal suppository containing *Lacttiplantibacillus pentosus* KCA 1 for treatment of bacterial vaginosis:Can Fourier Transform-Infrared (FT-IR) spectroscopy be used for identification of *Lactiplantibacillus pentosus* KCA1. *Journal of Medical Laboratory Science* 31 (2): 52-72.

Holt JG, Krieg NR, Sneath PHA et al (1994). Bergey's Manual of Determinative Bacteriology, 9th ed. Williams & Wilkins, Baltimore, pp. 175–189

Holzapfel WH, Wood BJB (2014). Lactic Acid Bacteria: Biodiversity and Taxonomy. Wiley-Blackwell, pp. 249–353

Jones SE, Versalovic J (2009). Probiotic *Lactobacillus reuteri* biofilms produce antimicrobial and anti-inflammatory factors. *BMC Microbiology 9:* 35.

Kumar A, Kolay A, Havelikar U (2023) Modern aspects of suppositories: A review. *European Journal of Pharmaceutical Research* **3**(4): 23–29.

Li L, Qiu X, Lu S et al (2026). The role of probiotics *Limosilactobacillus euteri*, *Ligilactobacillus salivarius*, and *Lactobacillus johnsonii* in inhibiting pathogens, maintaining gut health, and improving disease outcomes. *International Journal of Molecular Sciences* 27(3):1545.

Luo Z, Chen A, Xie A et al (2023). *Limosilactobacillus reuteri* in immunomodulation: molecular mechanisms and potential applications. Frontiers in Immunology 9: 14, 1228754.

MacFaddin JF (2000). Biochemical Tests for Identification of Medical Bacteria, 3rd ed. Lippincott Williams & Wilkins, Philadelphia, pp. 57–345.

Melnyk G, Yarnykh T, Herasymova I (2020). Analytical review of the modern range of suppository bases. *Systematic Reviews in Pharmacy* 11(4): 503–508.

Shende S, Meshram B, Karemore H et al (2023). Development and characterization of glycerogelatin suppositories for enhancedefficacy. *European Journal of Pharmaceutical and Medical Research* 10(6): 522–528.

Shokryazdan P, Sieo CC, Kalavathy R et al (2014). Probiotic potential of *Lactobacillus* strains with antimicrobial activity against some human pathogenic strains. *BioMed Research International* 2014:927268

Spickler AR (2016). Enterohemorrhagic *Escherichia coli* and other *Escherichia coli* causing Hemolytic Uremic Syndrome. Iowa State University Center for Food Security Public Health, pp. 1–15.

Talarico TL, Dobrogosz WJ (1989). Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. *Antimicrobial Agents and Chemotherapy* 33(5):674-9.

Zhang T, Xue Y, Yue T et al (2020) Characteristics of aerobic vaginitis among women in Xi'an district: a hospital-based study. *BMC Women's Health* 20 (138):2-7.

CITATION Oloton E Yaluwu AE (2026) Influence of Glycerogelatin on the Antibacterial Activity of *Limosilactobacillus reuteri* CF48-3A Against *Escherichia coli.* Trend Nat Prod Res Vol 7(3):307-313. https://doi.org/10.61594/tnpr.v7i3.2026.177