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Evaluation of Polyethylene Glycol and Theobroma Bases on the Probiotic Activity of *Lactobacillus paragasseri* Against *Escherichia coli.*

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ABSTRACT

Background: *Escherichia coli* is a ubiquitous Gram-negative bacterium with pathogenic potential, necessitating alternative antimicrobial approaches. This study evaluated the comparative inhibition of *Escherichia coli* ATCC 25922 by *Lactobacillus paragasseri,* JV-V03 in combination with polyethylene glycol and *Theobroma* in co-culture systems.

Methods: Both microorganisms were characterized using phenotypic and biochemical techniques. Antibacterial activity was determined using the agar overlay method, while co-cultivation assessed comparative *E. coli* growth in combination with *L. paragasseri* JV-V03, polyethylene glycol, and Theobroma. Statistical significance was determined using two-way ANOVA.

Results: Characterization confirmed the identity of both microorganisms. The agar overlay method demonstrated bacteriostatic activity (IZD = 30.8 ± 0.62 mm). Co-cultivation reduced *E. coli* growth to 0.17 $\log_{10}$ CFU/mL/h (r = 0.728) in the presence of *L. paragasseri* JV-V03, indicating antagonism. Polyethylene glycol further enhanced the inhibitory activity, yielding 9.18×10^{-3} $\log_{10}$ CFU/mL/h ($r = 0.203$). In contrast, growth increased to 0.28 $\log_{10}$ CFU/mL/h ($r = 0.928$) in the *Theobroma* combination indicating a neutral effect. Differences were significant ($p < 0.001$) for survival with time. Tukey's HSD analysis showed polyethylene glycol significantly reduced *E. coli* ($p < 0.05$).

Conclusion: *L paragasseri* JV-V03 shows potential as a probiotic candidate for inhibiting *E. coli* ATCC 25922, while polyethylene glycol is an effective base for optimizing its antibacterial activity.

Keywords—*Escherichia coli* ATCC 25922, *Lactobacillus paragasseri* JV-V03, Polyethylene glycol, Probiotic antagonism, Theobroma base.

1. INTRODUCTION

Escherichia coli (*E. coli*) is a ubiquitous Gram-negative bacterium that predominantly colonizes the human and animal gastrointestinal systems. It can be both beneficial and pathogenic to human physiology. The harmful strains cause some infections, including gastrointestinal and urinary tract infections [1]. Specifically, this organism has played a major role in the propagation and maintenance of chronic inflammation in ulcerative colitis (UC) as shown by Pilarczyk-Zurek [2]. The gains from antibiotics were short-lived, as their widespread use and abuse eventually led to the emergence of resistant strains [3]. Extraintestinal pathogenic *Escherichia coli* (ExPEC) associated virulence genes were significantly prevalent in the UC group when compared to healthy control.
group [4]. Thus, the growing challenge of antibiotic resistance necessitates the development of effective alternative or adjunct therapeutic strategies. Attention has shifted toward the use of probiotic microorganisms as natural antagonists of pathogenic bacteria. *Lactobacillus gasseri* is generally one of the predominant lactic acid bacteria in the small human intestine and has been reported to contribute to immune regulation, the prevention of bacterial and viral infections, and the reduction of allergic symptoms [5]. Specifically, the *L. paragasseri JV-V03* contributes

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to cervicovaginal health by combining acidification, biofilm protection, and unique antimicrobial metabolites. This makes it a strong commensal species with notable antibacterial activity [6]. Generally, *Lactobacillus gasseri* has demonstrated growth-inhibitory effects against *Escherichia coli.* It could produce hydrogen peroxide (H_2O_2), which is known to inhibit *E. coli* [7,8]. The production of active bacteriocins can affect the development *of E. coli* [9]. Theobroma and polyethylene glycol bases are used for the formulation of suppositories. Theobroma base is a lipophilic base, while polyethylene glycol is a hydrophilic base [10]. An ideal base should maintain probiotic viability and antimicrobial activity while ensuring release of the incorporated organisms [11,12]. It is therefore important to choose bases that enhance optimal antibacterial activity in the host. Despite growing research on probiotic–pathogen interactions, limited information exists on the comparative influence of polyethylene glycol and Theobroma in the inhibitory activity of *Lactobacillus paragasseri* JV-V03 against *Escherichia coli* in co-culture systems. Understanding these interactions may provide valuable insight into choosing the most appropriate base for further formulation. Therefore, this study aimed to evaluate polyethylene glycol and Theobroma bases on the probiotic activity of *Lactobacillus paragasseri against Escherichia coli.*

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

The Biological materials comprised *Lactobacillus paragasseri* JV-V03, used as the probiotic strain, while *Escherichia coli* ATCC 25922, used as the indicator organism for antibacterial evaluation.

2.1.2 Chemical and Reagents

Chemical and reagents used are Mueller Hinton agar and Mueller Hinton broth (Titan Biotech Ltd, India), eosine methylene blue agar (Ready MED, (India), De Man-Rogosa-Sharpe, (MRS) Agar and broth (Titan Biotech, India), polyethylene glycol 6000 and polyethylene glycol 4000 (Qualikems, India), Theobroma base (Nigeria), 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).

2.1.3 Equipment and Other Materials

Equipment and other materials 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)

2.2. Methods

2.2.1 Phenotypic Characterization

The *L. paragasseri JV-V03* and *Escherichia coli* ATCC 25922 were characterized using phenotypic and biochemical techniques [13]. Gram reaction was assessed by Gram staining, while catalase activity was determined using hydrogen peroxide. Carbohydrate fermentation profiles 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 specifically for *Escherichia coli* ATCC 25922.

2.2.2 Agar overlay assay

The bacteriostatic activity of *L. paragasseri JV-V03* against *Escherichia coli* ATCC 25922 was determined using the Agar overlay method as described by Kaur and Sharma (2013) with modifications [14]. *L. paragasseri JV-V03* was cultured in de Man, Rogosa, and Sharpe (MRS) broth at 37 °C for 48 h. Fifty microliters (50 µL) of the culture was spotted onto the surface of 20mL solidified sterile MRS agar and incubated at 37 °C for 48 h to allow colonies to develop in microaerophilic conditions. The MRS agar plates containing confluent growth of *L. paragasseri* JV-V03 were thereafter overlaid with 10 mL of Muller-Hinton (MH) agar pre-inoculated with 100 µL of an 18 h culture of *E. coli* ATCC 25922. The overlaid plates were allowed to solidify and incubated for 18 h at 37 °C. The inhibitory zone diameter (IZD) values obtained were measured in triplicate and interpreted. Agar plugs from the inhibition zones were aseptically collected using a sterile inoculating loop and subcultured in freshly prepared nutrient broth. After incubation at 37 °C for 24 h, the absence of growth was regarded as bactericidal activity, while visible growth indicated bacteriostatic activity [15].

2.2.3 Cocultivation assay

The growth inhibition of *E. coli* ATCC 25922 was assessed by using the cocultivation method as previously described with specific modifications [16]. 6.5 X 10^5 CFU/mL of *E. coli* ATCC 25922 was incubated in a series of combinations with 2.3 X 10^5 CFU/mL of *L. paragasseri* JV-V03, Theobroma and polyethylene glycol base in MRS: MH broth. The following four experimental combinations were applied:

1. *E. coli* (control)
2. *E. coli* in combination with *L. paragasseri*
3. *E. coli* in combination with *L. paragasseri* and Theobroma base,
4. and *E. coli* in combination with *L. paragasseri* and polyethylene glycol (molecular weight 6000:4000).

Samples were collected at 4-hour intervals in duplicate over a 48 h period. Serial tenfold dilutions of all samples were prepared in phosphate buffer solution and plated on Eosin Methylene Blue agar for enumeration of *E. coli.*

2.3 Statistical Analysis

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

3. RESULTS

3.1 Effect of phenotypic characterization on microorganisms

Table 1 shows *L. paragasseri* strain JV-V03 was identified as a Gram-positive bacillus and catalase-negative. The organism 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 1: Phenotypic authentication of microorganisms

Test	Specific interactions	*L. paragasseri* JV-V03	*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	ND	+
	Voges-Proskauer	ND	-
	Citrate	ND	-
	Urease	ND	-

Key: GP = Gram -positive bacilli; GNB = Gram-negative bacilli; ND = Not done; + = Positive; - = Negative

3.2: Effect of antibacterial activity using agar overlay method

Table 2 shows *L. paragasseri* JV-V03 demonstrated antibacterial activity against *E. coli* ATCC 25922, with inhibition zone of 30.8 ± 0.62 mm. However, growth was observed during the bactericidal assessment.

Table 2: Antibacterial effects of *L. paragasseri* JV-V03 against *E. coli* ATCC 25922.

Organism	Bacteriostatic (IZD ± SD mm)	Bactericidal activity.
L. paragasseri JV-V03	30.8 ± 0.62	Growth observed

Key: IZD = Inhibition Zone Diameter; SD = Standard Deviation

3.3: Effect of antagonistic activity by cocultivation assay

Figure 1 illustrates the antagonistic effect of *Escherichia coli* ATCC 25922 by *Lactobacillus paragasseri* JV-V03 in the presence of polyethylene glycol and Theobroma base. The overall treatments were statistically significant ($p < 0.001$) over 48 h. Indicating that the different treatments produced significant changes in *E. coli*. The regression equation for *E. coli* was y =4.60 +0.24x ($r = 1.000$), while *E. coli* cocultivated with *L. paragasseri* was y= 4.54 +0.17x ($r = 0.728$). In the additional presence of polyethylene glycol, the regression equation was y =2.57 + 9.18x10 $^{-3}$x ($r = 0.203$), whereas in the presence of Theobroma base showed y = 3.61 + 0.28x ($r = 0.928$). Tukey HSD analysis showed that PEG treatment differed significantly from the other groups ($p < 0.05$)

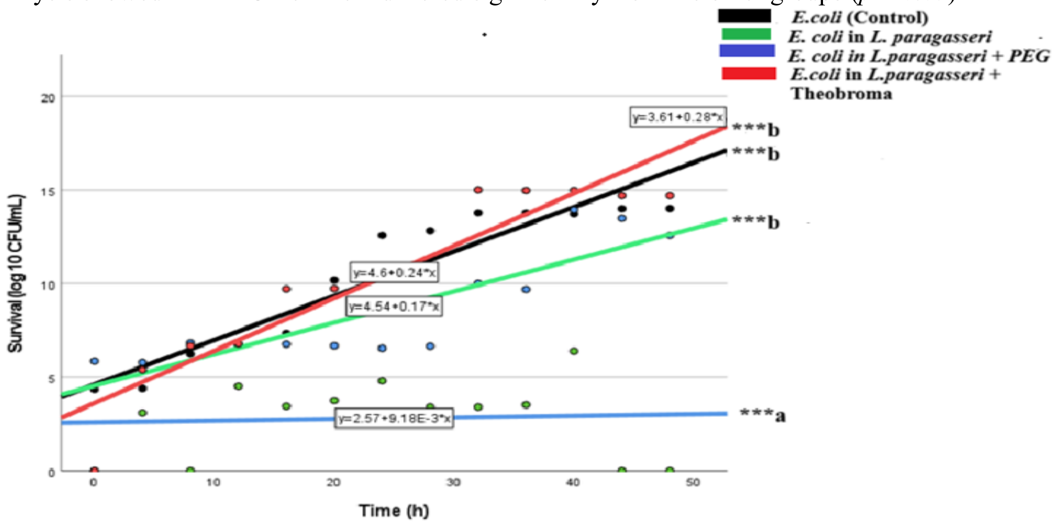

Figure 1: Inhibition of *E. coli* ATCC 25922 by *L. paragasseri* JV-V03 in presence of polyethylene glycol and Theobroma bases ($p < 0.001$); *E. coli* ($r = 1$); *E. coli* in *L. paragasseri* ($r = 0.728$); *E. coli in L. paragasseri* with PEG (r = 0.203) and *E. coli in L. paragasseri* with Theobroma ($r = 0.928$). Tukey's HSD analysis showed that PEG treatment (a), which differed significantly ($p < 0.05$) from the other groups (b), which did not differ significantly ($p > 0.05$).

3. DISCUSSION

The phenotypic identification test shown in Table 1 for *L. paragasseri* strain JV-V03 agrees with previous reports describing the biochemical profile of *L. gasseri* [17,18]. The observed characteristics, therefore, support the authentication of this *Lactobacillus* strain. Also, *E. coli* ATCC 25922 findings are consistent with the standard biochemical profile of *E. coli* reported in Bergey's Manual and other microbiological identification studies [19,20]. The phenotypic results obtained therefore validate the authentication of probiotic and pathogenic organism used in this study. *L. paragasseri* JV-V03 demonstrated strong antibacterial activity against *E. coli* ATCC 25922, as shown in Table 2. This finding indicates strong antagonistic activity based on IZD scaling [21]. However, growth was still observed during the bactericidal assessment, suggesting that the antibacterial activity was mainly bacteriostatic rather than bactericidal. The inhibitory activity may be due to the production of organic acids, hydrogen peroxide, and bacteriocin-like compounds, which are commonly associated with *Lactobacillus* species. Similar antibacterial effects of *L. gasseri* against enteric pathogens have previously been reported [22,23]. This outcome suggests that *L. paragasseri* JV-V03 possesses promising probiotic potential against *E. coli* ATCC 25922. The cocultivation assay showed variations in the growth dynamics of *E. coli* under different conditions as reflected by the regression equations and Pearson correlation coefficients displayed in Figure 1. The control *E. coli* ATCC 25922 culture showed a perfect positive linear growth relationship over time ($r = 1$) with a growth rate of 0.24 $\log_{10}$CFU/mL/h, indicating unlimited bacterial growth. The cocultivation with *L. paragasseri JV-V03* reduced the growth rate of *E. coli* ATCC 25922 to 0.17$\log_{10}$ CFU/mL/h with a strong positive growth relationship over time ($r = 0.728$). The antibacterial effect observed for *L. paragasseri* in this study agrees with previous findings. Showing inhibitory activity against *Escherichia coli*, attributed to organic acid production and other antimicrobial metabolites [7]. The strongest inhibitory effect was observed in *L. paragasseri* JV-V03 in combination with PEG base, where the growth rate was markedly reduced to $9.18 \times 10^{-3}\log_{10}$ CFU/mL/h the Pearson correlation coefficient decreased substantially ($r = 0.203$). The weak correlation indicates minimal increase in bacterial population over time, suggesting substantial suppression *of E. coli* growth under PEG base. Turkey HSD analysis further confirmed that PEG differed significantly. There is limited literature documenting this specific pathogen-*Lactobacillus*-base response under comparable experimental conditions. The enhanced antibacterial activity observed with PEG may be associated with its physicochemical characteristics, particularly its capacity to stabilize bioactive molecules and promote controlled diffusion to the target site [24]. This likely improved the retention and bioavailability of diffusible antibacterial metabolites produced by *L. paragasseri,* with inhibitory activity against *E. coli.* In contrast, *E. coli* ATCC 25922 in combination with *L. paragasseri* JV-V03 and Theobroma showed an increased growth rate of $0.28\log_{10}$ CFU/mL/h, with a very strong positive correlation ($r = 0.928$), indicating that the inhibitory activity of *L. paragasseri* was comparatively reduced in the presence of Theobroma. Published evidence describing this combined interaction under similar conditions remains limited. The reduced antibacterial activity observed in the Theobroma combination may be attributed to the limited release characteristics of fatty bases. Since drug release from Theobroma depends largely on partitioning into the aqueous phase, inefficient transfer of diffusible antibacterial metabolites may have reduced their effective concentration at the bacterial target site [25]. The highly significant two-way ANOVA result ($p < 0.001$) demonstrates that the interaction between base type (polyethylene glycol and *Theobroma*) and *L. paragasseri* JV-V03 significantly affected *Escherichia coli* ATCC25922 survivals over time. The Tukey's HSD analysis further strengthens the reduced growth rate in the PEG treatment when compared to other groups.

5. CONCLUSION

Lactobacillus paragasseri JV-V03 demonstrated significant inhibitory activity against *E. coli* ATCC 25922. Polyethylene glycol enhanced the antibacterial activity of the probiotic more effectively than the Theobroma base. These findings support the potential application of PEG-based probiotic formulations for managing *E. coli*-associated infections.

DECLARATIONS

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Conflict of Interest

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

Authors Contributions

Enosakhare Oloton conceived and designed the study, analyzed the data, and drafted the manuscript. Frances Chinaechekwa Madugba performed laboratory experiments and contributed to data collection. All authors read and approved the final version of the manuscript.

Ethical approval

Ethical approval was not required for this study because no human or animal subjects were involved.

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