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PARKIA SPECIOSA FRUIT EXTRACT ENHANCES *LACTOBACILLUS* COLONY FORMATION MEDIATED THROUGH MOTILITY

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ABSTRACT

Parkia speciosa known to contain various phytochemicals that can exert antimicrobial or growth-modulating effects, one out of that is anti-oxidant effects. In the present work *Parkia speciosa* fruit water extract was collected and added with *Lactobacillus* during fermentation process. Effect of *Parkia speciosa* fruit water extract on *Lactobacillus* colony growth behaviour mediated through bacterial motility was studied. It was observed that extract has significantly increased *Lactobacillus* colony growth behaviour and also increased bacterial motility very significantly.

1.0 INTRODUCTION

Plant extracts are known to contain various phytochemicals that can exert antimicrobial or growth-modulating effects. The interaction between *Lactobacillus* and *Parkia speciosa* extract was studied to determine whether the extract had any inhibitory, stimulatory, or neutral effect on the probiotic bacteria. This step was particularly important because while natural extracts may be effective against pathogenic bacteria, they should ideally not harm beneficial microorganisms. The experiments involved introducing the plant extract into *Lactobacillus* cultures and monitoring changes in CFU counts and motility patterns. Observations from this stage provided insights into the compatibility of plant-derived compounds with probiotic bacteria, which is a crucial factor in developing safe and effective natural therapies.

Understanding such interactions helps ensure that antimicrobial treatments do not disrupt the balance of beneficial microbiota. In the final phase of the study, the research progressed towards analyzing the interaction between beneficial and pathogenic bacteria by combining *Lactobacillus* and *Escherichia coli* cultures. This co-culture approach aimed to simulate natural microbial environments, such as those found in the human gut, where multiple microorganisms coexist and interact. Studying these interactions is essential for understanding microbial competition, antagonism, and possible synergistic effects. Experiments were conducted both individually and in combination to compare the growth dynamics, CFU counts, and motility of each organism. The co-culture studies revealed how *Lactobacillus* may influence the growth and behaviour of *E. coli*. It is well established that *Lactobacillus* produces lactic acid and other antimicrobial substances such as bacteriocins, which can inhibit the growth of pathogenic bacteria. Therefore, observing reductions in CFU or changes in motility in mixed cultures provided evidence of antagonistic interactions.

Adhesion in *Lactobacillus* is primarily mediated by surface proteins, lipoteichoic acids, and exopolysaccharides that enable the bacteria to bind strongly to surfaces once contact is established. Although they lack active movement, these bacteria are highly efficient in adhering to biological surfaces such as intestinal mucosa due to specific receptor-ligand interactions. In some rare cases, certain strains of *Lactobacillus* may show limited motility, but this is not a dominant factor in their adhesion process. Instead, their ability to form stable biofilms and produce extracellular substances plays a more significant role.

In the present work we have examined the effect of *Parkia speciosa* fruit water extract in modulating *Lactobacillus* cell count in colony behaviour mediated through bacterial motility.

2.0 MATERIALS AND METHODS

2.1 Demonstration of bacteria

At first take out the bacteria culture, from the incubator and keep the mother culture in laminar air flow unit. Then Aliquot 50 µl of culture and place over one end of clean grease free slide. By the help of another clean slide, prepare a smear over the slide. After that keep the slide over flame of a burner for 5-7 sec. During that time cell will fix over the glass slide. Then add 3-4 drops of crystal violet over the slide or flood the slide with crystal violet for 30 sec. After that wash the slide with distilled water to remove all excess dye over the slide. Air dry the slide at room temperature. Flood the slide with iodine solution for 5-10 seconds. Then wash gently again with water. Allow the slide to dry at room temperature. Then flood the slide with alcohol for 15 sec. After that wash the slide with distilled water to remove all excess dye over the slide. Air dry the slide at room temperature. Then add 3-4 drops of safranin dye over the slide or flood the slide with crystal violet for 30 sec. After that wash the slide with distilled water to remove all excess dye over the slide. Air dry the slide at room temperature. At last observe the slide under microscope first at 10x followed by that upto 40x magnification.

2.2 Preparation of bacterial culture media (nutrient broth)

The required amount of nutrient broth powder is weighed and dissolved in distilled water in a conical flask with continuous stirring to ensure complete mixing. The pH of the medium is adjusted to neutral (around 7.0) using a pH meter or pH paper. The flask is then plugged with cotton, covered with aluminum foil, and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the broth is allowed to cool and is then ready for inoculation of bacterial cultures such as *Lactobacillus* and *Escherichia coli.*

2.3 Preparation of bacterial culture media (nutrient agar)

The required quantity of nutrient agar powder is weighed and mixed with distilled water in a conical flask, followed by heating to dissolve the agar completely. The pH is adjusted to neutral, and the flask is plugged with cotton and covered with aluminum foil. The medium is sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the molten agar is allowed to cool slightly and then poured aseptically into sterile Petri plates inside a laminar airflow cabinet. The plates are left undisturbed until the agar solidifies, forming a suitable surface for bacterial growth.

2.4 Subculture of bacteria in nutrient broth

Under aseptic conditions, the inoculating loop is sterilized by flaming and allowed to cool. A small amount of bacterial culture is picked from the stock culture and transferred into sterile nutrient broth. Both Lactobacillus and E. coli are inoculated either separately or together to prepare mixed culture samples. The inoculated broth tubes are incubated at an appropriate temperature (usually 37°C) for 18–24 hours to allow bacterial growth, indicated by turbidity in the broth.

2.5 Subculture of bacteria and measurement of CFU

A small volume of bacterial culture is taken and spread evenly onto the surface of nutrient agar plates using a sterile spreader. For CFU determination, serial dilution of the culture is performed, and appropriate dilutions are plated to obtain countable colonies. The plates are incubated at 37°C for 24 hours. After incubation, visible colonies are counted, and the number of colony forming units (CFU) is calculated based on the dilution factor. This helps in estimating the viable bacterial population in both individual and mixed cultures.

2.6 Study of Lactobacillus motility

The motility test is performed by inoculating the semi-solid agar medium with the mixed bacterial culture using a sterile inoculating needle by stabbing vertically into the medium. The tube is incubated at 37°C for 24 hours. After incubation, the pattern of growth is observed; diffuse growth spreading away from the stab line indicates motility, while growth restricted to the stab line indicates non-motility. Alternatively, a hanging drop method may be used, where a drop of culture is observed under a microscope to detect movement. This helps in studying the motility behavior of *Lactobacillus* in the presence of *E. coli* method.

3.0 RESULTS

In the present work *Lactobacillus* growth behaviour was observed from bacterial colony forming units formed over agar media. CFU count (**Table 1**) and respective bacterial colony plate was reported in the present work (**Figure 1**).

Table 1: CFU count data of *Lactobacillus* in absence and presence of *Parkia speciosa* fruit water extract.

Sample	CFU count in absence of Parkia extract	CFU count in presence of Parkia extract
Yogurt	70	269
Yogurt	58	254
Yogurt	47	232

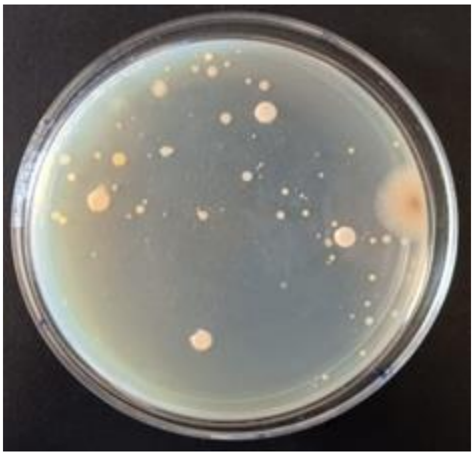

Figure 1: Colony growth (CFU count) of *Lactobacillus* in absence of *Parkia speciosa* fruit water extract.

Further *Parkia speciosa* fruit water extract was collected and studied its effect on growth behaviour of *Lactobacillus*. It was observed that after addition of *Parkia speciosa* fruit water extract, CFU count of *Lactobacillus* was significantly increased (**Table 1**). Such observation were reported and compared with respect to CFU of untreated samples (**Figure 2**).

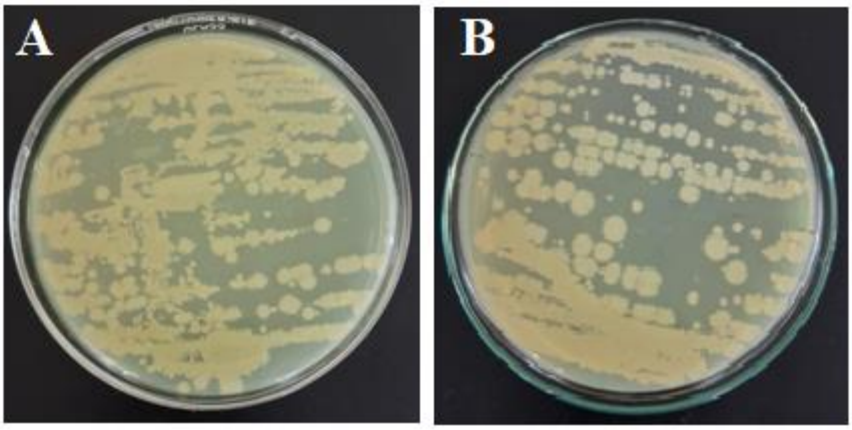

Figure 2: After treatment with *Parkia speciosa* fruit water extract, the *Lactobacillus* plates showed enhanced colony growth. The treated plates displayed larger, more expanded colonies, indicating that the extract promoted bacterial growth rather than inhibiting it. This suggests that *Parkia speciosa* extract may act as a growth enhancer or nutrient source for *Lactobacillus*.

Furthermore, we have studied effect of *Parkia speciosa* fruit water extract on *Lactobacillus* motility behavior. It was observed that after addition of *Parkia speciosa* fruit water extract bacterial motility has significantly increased. This observation suggests that the extract exhibits more uniformly scattered colonies across the plate from the centre of inoculation, indicating moderate motility with no strong directional spread.

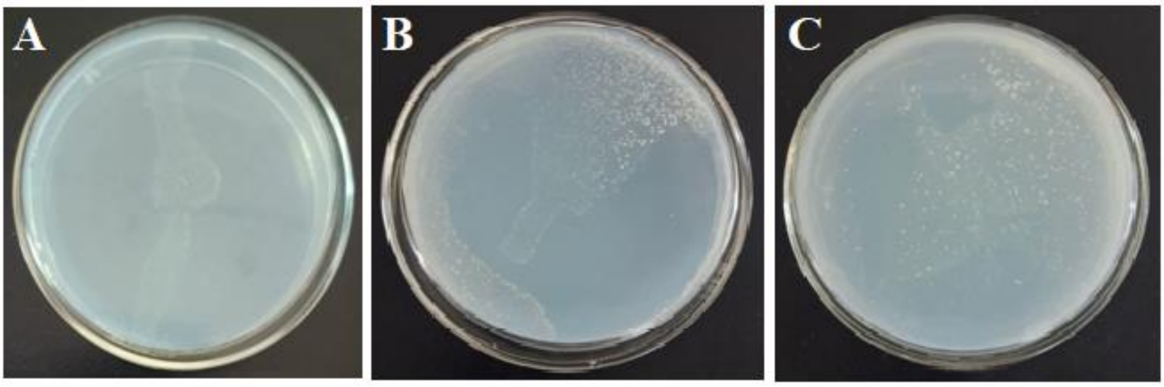

Figure 3: [A] Motility Plate of *Lactobacillus* without treatment of *Parkia speciosa* fruit water extract, indicating active, very slow bacterial movement and less motility.

[B] Motility Plate of *Lactobacillus* after treatment with *Parkia speciosa* fruit water extract displays scattered colonies with less dense spreading, suggesting moderate motility compared to untreated sample.
[C] Motility Plate of *Lactobacillus* after treatment with *Parkia speciosa* fruit water extract exhibits more uniformly scattered colonies across the plate from the centre of inoculation, indicating very high multidirectional motility.

4.0 CONCLUSION

The significance of this research lies in its contribution to the development of alternative fortification strategies of yogurt that is not only effective but also environmentally sustainable and compatible with beneficial microbiota. The findings emphasize the importance of utilizing natural resources such as plant extracts, alongside probiotic bacteria, to address current challenges in infection control and antibiotic resistance. By bridging the gap between traditional natural remedies and modern microbiological techniques, this work lays the foundation for future advancements in the field of antimicrobial research and probiotic applications.

COMPETING INTEREST

All authors declare that they have no competing interests.

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