Evaluation of antibacterial activity of probiotic *Bifidobacterium longum* and *Saccharomyces cerevisiae* against *E. coli O157:H7*

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Abstract:

This research was directed to assess the antibacterial activity of the probiotic strains *Bifidobacterium longum* and *Saccharomyces cerevisiae* against *Escherichia coli* O157:H7, a pathogenic strain responsible for severe foodborne illness. The study employed three methods spot method, agar diffusion and vertical line method to evaluate the antibacterial action of *B. longum* and *S. cerevisiae*. *Bifidobacterium longum* exhibited considerable antibacterial activity, with noticeable inhibition of *E. coli* H7: O157 growth in comparison to S. cerevisiae. Despite the fact that *S. cerevisiae* showed antibacterial properties, its inhibition activity was not as much as than those of *B. longum.* The study asserts that *Bifidobacterium longum* showed a remarkable antibacterial effect against *E. coli* H7: O157, proposed its suggesting probable application as an agent to prohibit or control this pathogen infection. *Saccharomyces cerevisiae* additionally displayed antibacterial effects, although it is less efficient. However, the combination of both of them exhibited remarkable inhibition. These findings will be paved the way of understanding the influence of probiotics in infection control and food safety.

Key words: antibacterial activity, probiotic *Bifidobacterium longum*, Saccharomyces *cerevisiae*, *E. coli O157:H7*

Introduction:

Probiotics are live microorganisms that converse health reimbursements to the human when directed in acceptable quantities **[1]**. Apart from their well-known effects in digestive health and immune modulation, latest research has explored their possible as antimicrobial components against pathogens such as bacteria, fungi and viruses **[2,3]** including strains of *E. coli* O157:H7 **[4]**.

Many studies investigated the contrivances of probiotics antimicrobial activity due to the dilemma of antibiotic resistance of pathogenic bacteria **[5]**. Probiotics directed the antimicrobial activity through variable mechanisms **[6]**. The primarily mechanism is the production of antimicrobial substances such as organic acids, bacteriocins, and hydrogen peroxide. These substances can constrain the growth of pathogens by disorderly cellular progressions or directly destructive pathogenic membranes **[7]**. Moreover, probiotics contest with pathogens for adhesion locations on intestinal epithelial cells, thus stopping settlement and succeeding infection **[8]** (Iqbal et al., 2021).

In vitro researcher's work and some clinical studies have delivered valued visions into the antimicrobial possible of probiotics against pathogenic bacteria **[9,10,11]**. For example, research conducted by **[12]** verified that *Lactobacillus* strains isolated from human gut microbiota expressively reduced the growth and feasibility of *E. coli* O157:H7in imitation gastrointestinal environments. These conclusions highlight the strain-specific differences in probiotic effectiveness and highpoint the significance of choosing suitable strains for therapeutic applications **[13]**. The most applicable probiotics are genus of the *Lactobacilli* group, which has lately been separated into many species *Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus crispatus, Lactiplantibacillus plantarum Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, Lacticaseibacillus casei, Lactobacillus gasseri Limosilactobacillus reuteri, Levilactobacillus brevis, Ligilactobacillus* and others.

The second genera are *Bifidobacterium* which include *Bifidobacterium animalis* subsp. *infantis*, *Bifidobacterium bifidum*, *Bifidobacterium longum* and even certain strains from some yeasts (e.g., *Saccharomyces cerevisiae* var. *boulardii*) nominated as probiotics **[14]**.

Bifidobacterium longum habitat in the human gut, generated chemical components and organic acids such as lactic acid and acetic acid, forming acidic surroundings that reduce the growth

of pathogenic bacteria like *E. coli* **[15]**. Also, *Bifidobacterium longum* has been detected to display antimicrobial action by producing some peptides that directly object and disturb the cell membranes of *E. coli* H7 0157 **[16]**.

Saccharomyces cerevisiae, a yeast species has many applications in food fermentation and in probiotic food supplementation **[17]**, also displays antimicrobial capability against many pathogenic bacteria. This yeast constructs various compounds such as organic acids, ethanol, and antimicrobial peptides that enhance the inhibitory approach against pathogens **[18]**. The probiotic potential and antimicrobial activity of *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae*, and *Bifidobacterium longum* were investigated against some foodborne pathogenic bacteria such as *E. coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes*. The study demonstrated that *L. plantarum* and *B. longum* revealed antimicrobial action against *E. coli O157:H7*, *S. typhimurium* and *L. monocytogenes*. Conversely, *S. cerevisiae* did not display inhibition to any of the seleceted pathogens **[4]**.

Materials and Methodology

1. Strain Selection, Culturing and Preparation of *E. coli* H70157 Inoculum:

Strains of *Bifidobacterium longum* was kindly obtained from bacteriology laboratory /Faulty of sciences/Kufa University. Cultures are maintained under anaerobic conditions in MRS (De Man, Rogosa, and Sharpe) broth at 37 °C, while *Saccharomyces cerevisiae* strains used in in this study were isolated from fermented products in food sciences laboratory /Faculty of Agriculture /Kufa University. Yeast cultures are grown aerobically in YPD (Yeast Peptone Dextrose) broth at 26 °C. Pathogen strain *E. coli* O157:H7 was obtained from Karbala Food Safety and Medical Center. This strain was used as indicators of antibiotic activity. The culture was activated from glycerol stock by culturing in nutrient broth media overnight at 37°C.

2. Antimicrobial Assays:

1. **Spot method**

   Antibacterial activity was investigated by an agar spot test using a colony overlay assay **[18]**. The overnight cultures (10^5–10^9 CFU/mL) of B. *longum* were spotted (5µL) on the

surface of MRS agar plates and incubated at 37 °C under anaerobic conditions for 24 h. Similarly, 5 µl of *S. cerevisiae* was spotted on SB agar plates and incubated at 26 °C aerobically for 24 h. After incubation, the plates were overlaid with 10 mL of nutrient agar, previously inoculated with 100 µL (10^6–10^9 CFU/mL) of an overnight culture of the indicator pathogen strain *E. coli* O157:H7. The examination was performed in triplicates.

2. Agar Well Diffusion method

This method involved pouring nutrient agar plates and inoculating them with a lawn of *E. coli* H70157. The cultures of (*Bifidobacterium longum* or *Saccharomyces cerevisiae*) are added to the wells eighter each strain alone or in combination. After incubation, zones of inhibition around the wells indicate antimicrobial activity against *E. coli* O157:H7, measured as the diameter of clear zones. Diameter of the zone of inhibition around the colony was examined and measured using a ruler. Inhibition zone with a diameter of 6 mm or larger was considered as positive inhibition **[20].** The examination was performed in triplicates.

2. Vertical line method

A single line of Bifidobacterium longum and *Saccharomyces cerevisiae* was inoculated and placed vertically to the streaked *E. coli O157:H7*bacteria using a sterile inoculating loop. The plates were inverted and incubated at the appropriate temperature 37°C for the bacteria and 18-24 for the yeast. After incubation, the plates were observed for zones of inhibition or growth patterns. The areas of inhibition of pathogenic bacteria's growth were determined and measured.

RESULTS AND DISCUSSION

The results of tests on the antagonistic activity of young cultures of *Bifidobacterium longum* and bread *Saccharomyces cerevisiae*, individually and in combination, against the selected *E. coli* O157:H7 bacteria under study showed that there is resistance to different degrees using the hole method, the vertical line method, and the spot method (Table 1). It was found that the hole method was ineffective in showing the biological activity of bacteria and yeast individually or in

combination, and even in the control sample, no growth inhibition was observed when using the same method, as shown in the tables below.

Table1: Average diameter of the inhibition zone for bacterial growth for *Bifidobacterium* bacteria

Average diameter of the bacterial growth inhibition zone (mm) for Bifidobacterium			**Microbial isolates**
Vertical line method	**Spot method**	**Agar Well Diffusion method**	
20	**20**	**0**	**E. coli O157:H7**
23	**22**	**0**	**E. coli control sample**

It is clear from the table above that the average diameter of inhibition by the vertical line method for *Bifidobacterium* bacteria against *E. coli* O157:H7 bacteria is 20 mm, while the average diameter of inhibition for the control sample was 23 mm. while for the spot method the inhibition zone of the pathogenic strain (20mm) and the control (22mm). no inhibition zone was observed in both pathogenic strain and the control in the hole method.

The inhibitory effect of *Bifidobacterium* against *E. coli* O157:H7 was lower with respect to that observed against *E. coli*. The differences observed between the results obtained from the different methods could be due to possible physicochemical interactions between the active metabolites produced by the bacteria and the medium nutritional. This was consistent with what was previously achieved **[21]**, where (the aim of the study was to verify the ability of both Bifidobacterium and *Lactobacillus* bacteria, alone or in combination, to inhibit the growth of pathogenic gram-negative and gram-positive bacterial strains and some fungi using different methods. Cell-free supernatants were obtained by centrifugation and filtration from single or mixed broth cultures and the inhibitory activity was tested using both agar diffusion and dilution methods. In order to obtain some preliminary information about the chemical nature of the active metabolites released in the supernatants. The highest inhibitory activity was demonstrated by the untreated supernatant obtained directly from the both cultures.

Table (2) shows that yeast has a lower inhibitory effect than what was observed on *Bifidobacterium* bacteria, at a rate of 17 and 19 for the pathogenic bacteria and for the control

bacteria, respectively. These results were similar to the results obtained by **[22]**. In their study, the continuous culture conditions did not yield any effective inhibitory substances against *E. coli* O157:H7, despite the possibility that they were produced by *S. cerevisiae* subsp. boulardii in a feed supplement containing this yeast.

Table (2): Average diameter of the inhibition zone for bacterial growth of *Saccharomyces cerevisiae.*

Average diameter of the bacterial growth inhibition zone (mm) for Saccharomyces cerevisiae			**Microbial isolates**
Vertical line method	**Spot method**	**Agar Well Diffusion method**	
17	**10**	**0**	**E. coli O157:H7**
19	**14**	**0**	**E. coli control sample**

As for the use of bacteria and yeast combined as natural antibiotics (Table 3), the antagonistic effect was effective and clear inhibition in both the spot and vertical line methods against *E. coli* O157:H7 bacteria and control sample, which gives an indication that the use of these microbial agent's combination could be promising for alleviating severe effects as a result of infection with this type of bacteria, such as acute diarrhea.

Table 3: Average diameter of the inhibition zone for bacterial growth for *Bifidobacterium* bacteria and *Saccharomyces cerevisiae* yeast combined.

Average diameter of the bacterial growth inhibition zone (mm) for Bifidobacterium and Saccharomyces cerevisiae combined			**Microbial isolates**
Vertical line method	**Spot method**	**Agar Well Diffusion method**	
25	**22**	**0**	**E. coli O157:H7**
20	**20**	**0**	**E. coli control sample**

According to a recent overview, gastrointestinal infections in particular diarrheal diseases are one of the leading causes of morbidity and mortality worldwide. Although treatment with antibiotics has led to significant improvements in health, their overuse is associated with the development and dissemination of specific resistance mechanisms, contributing to the antimicrobial resistance emergency due to the death of more than 700,000 patients globally each year. Source: Imbalance has been documented. Balance between major microbial populations distributed in the human intestine in patients with gastrointestinal and urinary tract infections **[23]**.

Several studies have shown that *Bifidobacterium* and *Lactobacillus* are able to competitively exclude pathogenic bacteria and yeasts, either directly, through interactions with pathogenic strains, or indirectly, through the production of active metabolites and stimulation of the host's immune defense. Therefore, they can Probiotics represent a potential alternative to conventional antimicrobials either as prevention or as treatment for gastrointestinal infections and for these reasons remain one of the main means of comparing these infections **[24,25].** The strains, currently used as probiotics, belong to the genera *Bifidobacterium* and Lactobacillus, which are commonly found in the human intestinal microbiota and are capable of producing antimicrobial metabolites such as organic acids, hydrogen peroxide, ethanol, diacetyl, acetaldehyde, saturated or trans-free fatty acids and other compounds such as Peptides and bacteriocins. **[26,27]**.

CONCLUSION

To conclude with, this research was evaluated the antibacterial activity of *Bifidobacterium longum* and *Saccharomyces cerevisiae* against *Escherichia coli* O157:H7. The findings revealed definite differences in their activity. *Bifidobacterium longum* exhibited a considerable antibacterial action of inhibiting the of E. coli O157:H7 growth in the spot and vertical line methods. However, no inhibition was observed while the agar well diffusion method.

In comparison with, *Saccharomyces cerevisiae* exhibited some level of antibacterial activity in vertical and spot methods (17 and 10 mm) respectively, but was less effectual in contrast to *B. longum*. The combination *Bifidobacterium longum* and *Saccharomyces cerevisiae* showed significant inhibitory effect more than using each strain individually. These outcomes highlight the potential of probiotic *B. longum* as a promising candidate for probiotic-based therapies targeted and controlling the infections caused by E. coli O157:H7. Further research is required to examine

the mechanisms behind *B. longum and Saccharomyces cerevisiae* efficacy and to assess their applications in clinical and food safety layouts.

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