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Probiotics and Antimicrobial Effect of *Lactiplantibacillus plantarum, Saccharomyces cerevisiae,* and *Bifidobacterium longum* against Common Foodborne Pathogens in Poultry

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Abstract: The probiotic potential and antimicrobial activity of *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae*, and *Bifidobacterium longum* were investigated against *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes*. Selected strains were subjected to different acid levels (pH 2.5–6.0) and bile concentrations (1.0–3.0%). Strains were also evaluated for their antimicrobial activity by agar spot test. The potential probiotic strains tolerated pH 3.5 and above without statistically significant growth reduction. However, at pH 2.5, a significant ($p < 0.05$) growth reduction occurred after 1 h for *L. plantarum* (4.32 log CFU/mL) and *B. longum* (5.71 log CFU/mL). *S. cerevisiae* maintained steady cell counts for the entire treatment period without a statistically significant ($p > 0.05$) reduction (0.39 log CFU/mL). The results indicate at 3% bile concertation, 1.86 log CFU/mL reduction was observed for *L. plantarum*, while *S. cerevisiae*, and *B. longum* growth increased by 0.06 and 0.37 log CFU/mL, respectively. *L. plantarum* and *B. longum* demonstrated antimicrobial activity against *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes*. However, *S. cerevisiae* did not display any inhibition to any of the pathogens. The results indicate that *L. plantarum* and *B. longum* present probiotic potential for controlling *E. coli* O157:H7, *S.* and *L. monocytogenes* in poultry.

Keywords: probiotics; poultry; *L. plantarum*; *S. cerevisiae*; *B. longum*; acid tolerance; bile tolerance; antimicrobial inhibition

1. Introduction

The poultry industry is slowing turning away from antibiotics, owing to the rising public health concern over antibiotic-resistant pathogens. The imprudent use of antibiotics in the poultry industry is associated with the development of antimicrobial resistance [1,2]. Antimicrobial resistance in food animals is a major concern due to the potential dissemination of resistant pathogens to humans via the food chain [3]. The extensive use and misuse of antibiotics in animal farming have contributed to the emergence and spread of antibiotic-resistant *Salmonella*, *Campylobacter*, and *Listeria monocytogenes* [4–6]. Additionally, the use of antibiotics in animal production make food unsafe due to the accumulation of residues in edible tissue [7,8]. These residues in meats have been reported to cause allergies in hypersensitive consumers [9]. Antibiotics used at sub therapeutic doses have been restricted in many countries including the EU and USA [10,11].

The ban of antibiotics as antibiotic growth promoters (AGPs) has economically impacted the livestock production systems due to diseases [12]. Antimicrobial resistance and the ban of AGPs have driven the poultry industry to search for substitutes with comparable benefits to antibiotics [13]. Novel choices for antibiotics are necessary to satisfy the increased consumer demand for safe poultry products [14] and to mitigate antimicrobial resistance in the food chain. Probiotics have shown many beneficiary effects in poultry including growth performance [15,16], improved meat quality and flavor [17,18], and the reduction of pathogenic microorganisms [13,19].

Lactobacillus acidophilus, *Lactobacillus casei*, *Bacillus* spp., *Bifidobacterium bifidum*, *Lactococcus lactis*, and *S. cerevisiae* have been used as probiotics [20,21]. These strains have beneficial effects including broiler performance [22–24], the balance of intestinal microflora, and pathogen inhibition [24,25]. However, the effectiveness of the probiotics largely depends on their viability in the harsh gut environment of poultry [16]. Probiotics in the gastrointestinal tract are compromised by its high acidic and bile salt environment, which could ultimately reduce viable cells to utilize their desired functions [26]. Probiotics utilize their antimicrobial activity by producing antimicrobial substances including bacteriocins, organic acids, antimicrobial peptides, competitive exclusion, and modulating the host immune system [27–31]. *Lactiplantibacillus plantarum* is among the most frequently used probiotics [32].

It has been documented that bacteriocins-like compounds are responsible for the antimicrobial property of *Bifidobacterium* [28,33,34]. *Bifidobacterium* is a well known probiotic for its inhibition ability against many pathogens including *Escherichia coli* O157:H7, *Salmonella typhimurium, L. monocytogenes*, and *Staphylococcus aureus* [35]. Lactic acid bacteria (LAB) and yeasts have also been reported for their antimicrobial effect against many pathogens [36–40].

Although probiotics are promising substitutes for antibiotics, there has been several studies that report the inability of several well known probiotics to inhibit the growth of pathogens, for example, *Bifidobacterium* strains have failed to display antimicrobial activity against *E. coli* K-12 and *Salmonella enterica* serovar Typhimurium American Type Culture Collection (ATCC) 14,028 [41]. *S. cerevisiae* has also been reported not to inhibit the growth of *E. coli* O157:H7, *S. enterica* serovar Typhimurium, and *Salmonella paratyphi* [42]. Hence, it is important to evaluate the antimicrobial properties and ability of bacteria adaption to the host environment before their selection as probiotics. In the present study, we investigated the acid and bile tolerance of *L. plantarum*, *S. cerevisiae*, and *B. longum* and their ability to inhibit the growth of *E. coli* O157:H7, *S. enterica* serovar Typhimurium, and *L. monocytogenes*.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

In this study, *L. plantarum* (ATCC 8014), *B. longum* (ATCC 15708) and *S. cerevisiae* (ATCC 9763) were used as candidate probiotic strains for acid and bile tolerance studies. *L. plantarum* and *B. longum* were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and *S. cerevisiae* from Microbiologics Inc. (St. Cloud, MN, USA). All cultures were stored at −80 °C in 15% (*v*/*v*) glycerol and activated by two successive transfers in appropriate broth when needed. *L. plantarum* and *B. longum* were cultured in de Man Rogosa Sharpe (MRS) (Oxoid, Basingstoke, Hampshire, England) for 12–18 h at 37 °C anaerobically (; 80% nitrogen, 10% hydrogen and 10% carbon dioxide). *S. cerevisiae* was cultured in Sabouraud Dextrose (SB) (Becton, Dickinson and Company, Sparks, MD, USA) aerobically at 26 °C for 18 h. When needed, 100 µl each of *L. plantarum* and *B. longum* frozen culture was grown in 10 mL of MRS medium whereas *S. cerevisiae* was cultured in the SB medium.

2.2. Tolerance to Acidic pH Values

To test the acid tolerance levels of the strains, the method described by Jin et al. [43] was used. Briefly, 100 µL of each frozen strain was grown in 10 mL of its appropriate broth (*L. plantarum* and *B. longum* in

MRS Broth and *S. cerevisiae* in SB broth). *L. plantarum* and *B. longum* were grown anaerobically at 37 °C for 24 h, and *S. cerevisiae* was grown aerobically at 26 °C for 24 h. Subsequently, 100 µL viable cultures were transferred into freshly prepared 10 mL of their respective broth and incubated for another 24 h. The cultures were centrifuged at 3000 rpm for 10 min at 4 °C, the pellets were washed twice in sterile phosphate buffered saline (PBS), pH 7.2 (Sigma, St Louis, MO, USA) and suspended in 1 mL of PBS (Sigma). For each strain, 0.1 mL of culture suspension was added separately in tubes containing 2 mL of sterile PBS at various pH ranges from 2.5 to 6.0. Hydrochloric acid (2 M) was used to adjust the desired pH values of the PBS. Tubes containing PBS with tested strains were incubated anaerobically at 37 °C for *L. plantarum* and *B. longum* and aerobically at 26 °C for *S. cerevisiae*. During incubation, 1 mL of each culture in PBS was taken every hour for 5 h and the viable number of bacteria were enumerated by spread plate method. Approximately 0.1 mL of diluent was spread plated on MRS agar for *L. plantarum* and *B. longum* and SB agar for *S. cerevisiae*. After incubation, bacterial colonies were counted and recorded. The studies were performed in three independent experiments, and each assay was performed in triplicate to calculate intra-assay variation.

2.3. Bile Tolerance Test

Bile tolerance was tested using the methods described by Jin et al. [43] and Vernazza et al. [44]. *L. plantarum* and *Bifidobacteria longum* were cultured in MRS broth (Oxoid, Basingstoke, Hampshire, England) anaerobically at 37° C for 12 h. *S. cerevisiae* was cultured aerobically in SB. The *L. plantarum, B. longum,* and *S. cerevisiae* stored at −80 °C were sub-cultured twice in their respective media. Then, 100 µL from each strain was inoculated into 10 mL fresh tubes of the appropriate broth containing 0–3% bile salts (Oxoid, Basingstoke, Hampshire, England). A sample from each strain was taken every hour for 6 h and subjected to serial dilutions up to 10^{-8} dilutions, then 100µL of the selected diluent was spread plated onto the respective agar plates to calculate the CFU/mL. After the incubation period, viable bacterial colonies were counted. The bile tolerance assay was performed in triplicate with three replications per assay.

2.4. Antimicrobial Assessment

Antibacterial activity was investigated by an agar spot test by using a colony overlay assay described by Tejero-Sarinena et al. [45]. *E. coli* O157:H7 (ATTC 35150), *S. enterica* serovar Typhimurium (ATTC 13311) and *L. monocytogenes* (ATTC 19115) were used as indicators of antibiotic activity. *L. plantarum* and *B. longum* were cultured overnight in MRS broth at 37 °C in the anaerobic chamber, while *S. cerevisiae* was cultured aerobically in SB broth at 26 °C. Then, overnight cultures (10^7–10^9 CFU/mL) of *L. plantarum* and *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 0.7% (*w*/*v*) nutrient agar, previously inoculated with 100 µL (10^7–10^9 CFU/mL) of an overnight culture of the indicator pathogen strains (*E. coli* O157:H7, *S. enterica* serovar Typhimurium and *L. monocytogenes*). All the plates were incubated aerobically at 37 °C for 24 h. Diameter of the zone of inhibition (ZOI) around the colony was examined and measured using a ruler. A ZOI with a diameter of 5 mm or larger was considered as positive inhibition [46]. This assay was performed in triplicates.

2.5. Statistical Analysis

Data analysis was carried out by using Duncan's multiple range test to define the mean differences between the specific treatments. $p < 0.05$ was considered to indicate a significant difference. All analyses were conducted using SAS software (version 9.1, 2004; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Acid Tolerance

L. plantarum reduction with the progression of time (0–5 h) was not statistically significant ($p > 0.05$) at pH 3.5 to 6.0. The growth reduction recorded for a 5 h treatment was 0.40, 0.25, 0.44, 0.20, 0.35, 0.06 log CFU/mL at pH 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0, respectively. This insignificant reduction indicates high *L. plantarum* viability when cultivated at 3.5 to 6.0 pH range. However, at pH 2.5, the growth of *L. plantarum* significantly ($p < 0.05$) reduced (4.32 log CFU/mL) at 1 h, and no viable cells were detected from 2 to 5 h. However, *L. plantarum* successfully survived for 3 h without losing a significant number of viable cells at pH 3.0 as demonstrated by 1.30 log CFU/mL growth reduction from 0 to 3 h. Eventually, at pH 3.0, statistically significant ($p < 0.05$) growth reduction (4.80 log CFU/mL) was observed from 3 to 4 h. No detectable *L. plantarum* viable cells were observed after 5 h (Figure 1).

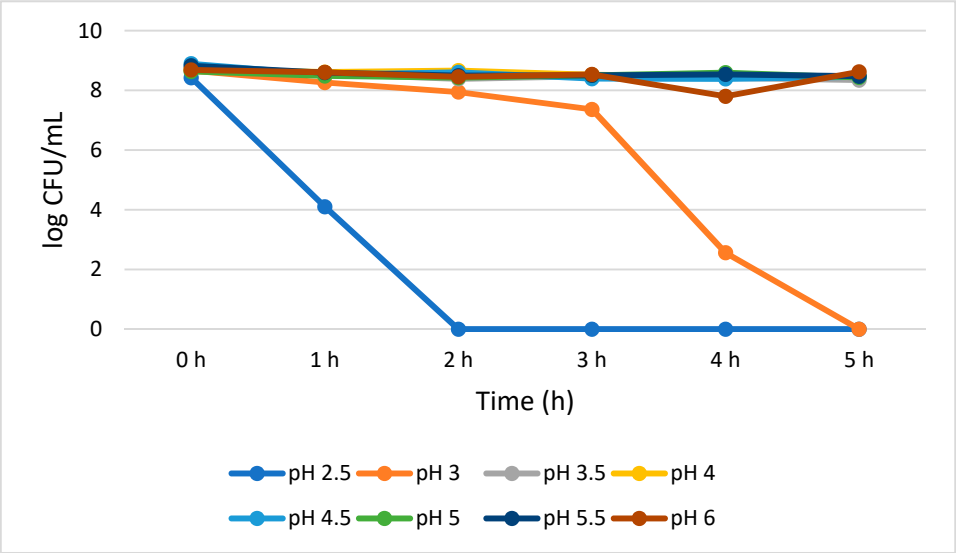

Figure 1. Acid tolerance of *L. plantarum* measured at different pH ranges from 2.5 to 6.0.

Figure 2 shows that *S. cerevisiae* demonstrated a better survivability than *L. plantarum* both at lower and higher pH values. *S. cerevisiae* showed consistent viability from 0 to 4 h with a 0.07 log CFU/mL growth reduction at pH 2.5. However, at pH 3.0, the growth of *S. cerevisiae* was insignificantly ($p > 0.05$) increased (0.15 log CFU/mL) during the 5 h treatment. At pH 4.0, 4.5, 5.5, and 6.0, the total viable cells was 6.26, 6.30, 6.25, and 6.18 log CFU/mL, respectively, at 5 h. At pH 5.0, a mixed trend on the total number of viable cells was observed, but overall, no significant growth reduction (0.02 log CFU/mL) was noted between 0 and 5 h.

The acid tolerance data for *B. longum* showed a significant ($p < 0.05$) growth reduction (5.71 log CFU/mL) when exposed for an hour at pH 2.5. At the same pH, an additional growth reduction of 1.25 log CFU/mL was also observed between 1 h and 2 h; however, from 3 h no viable cells were detected. At pH 3.0, the growth of the *B. longum* insignificantly decreased (0.70 log CFU/mL) from 0 h to 2 h, but suddenly significantly ($p < 0.05$) decreased by 2.99 log CFU/mL at 3 h, and no viable cells were detected at 5 h. Our results indicate that *B. longum* showed better stability at higher pH than lower pH environment. For example, after 5 h treatment at pH 4.0, 5.0, and 6.0, a steady number of population was observed and recorded as 8.47, 9.34, and 8.90 log CFU/mL, respectively, with no significant reduction in viable cells (Figure 3).

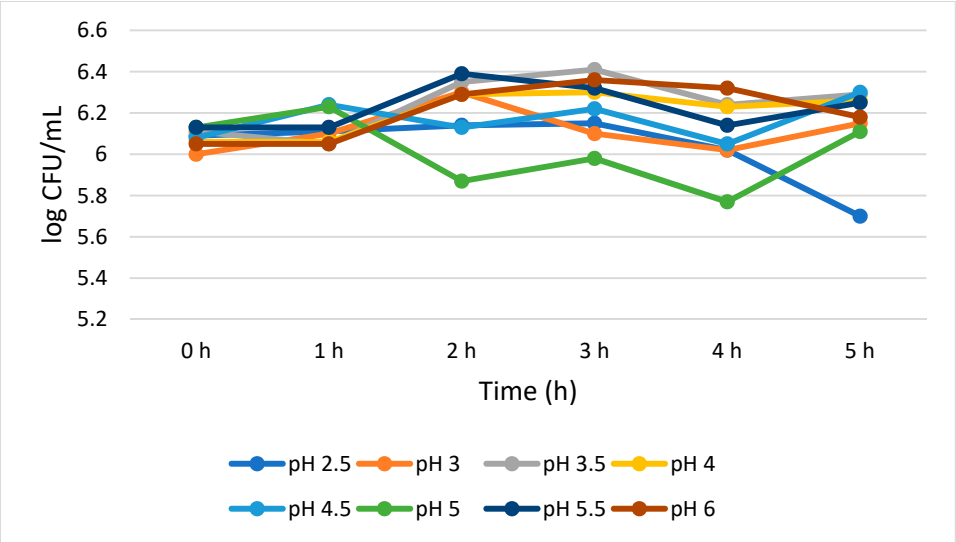

Figure 2. Acid tolerance of *S. cerevisiae* measured at different pH ranges from 2.5 to 6.0.

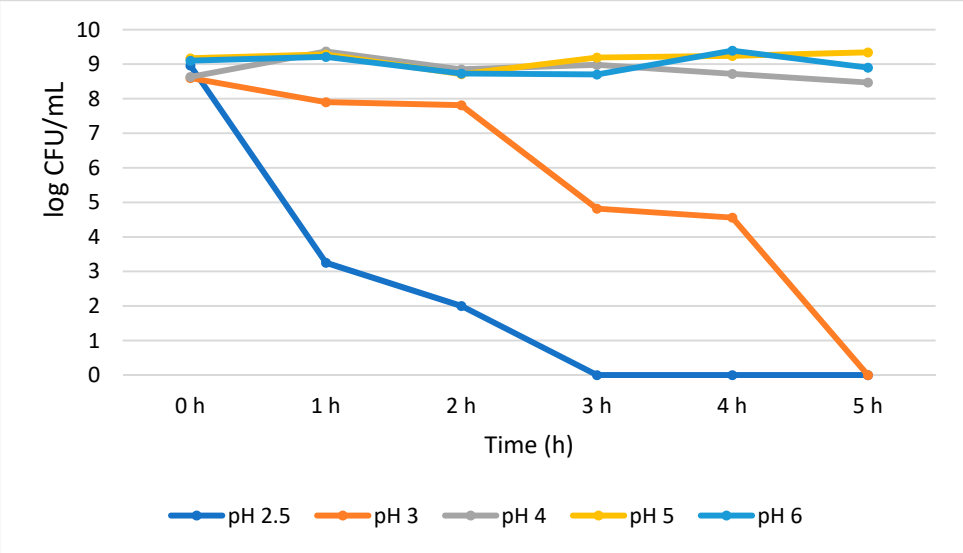

Figure 3. Acid tolerance of *B. longum* measured at different pH ranges from 2.5 to 6.0.

3.2. Bile Tolerance

Figure 4 shows that *L. plantarum* tolerated different concentrations of bile salt without compromising its growth during the 6 h treatment period. A 0.51 log CFU/mL growth increase ($p > 0.05$) was observed for the bile salt control (0%) at 6 h, while a not statistically significant (0.05) growth reduction of 0.25, 0.71, 0.88, and 1.86 log CFU/mL was recorded at 1.0%, 1.5%, 2.0, and 3.0% bile concentrations, respectively. Interestingly, in all the tested bile concentrations, the highest number of viable cells was observed at 5 h while the lowest was observed at 6 h.

S. cerevisiae's tolerance to bile salt was very similar to what was displayed in the case of *L. plantarum*. No statistically significant ($p > 0.05$) change in the growth rate was noticed from 1 to 6 h of treatment at different bile concentrations, rather an insignificant increase in growth rate was observed. The growth rate at 1.0, 1.5, and 3.0% bile concentrations was increased by 0.18, 0.82, and 0.06 log CFU/mL, respectively, from 1 to 6 h. However, at 2% bile concentration, the growth rate statistically significantly ($p < 0.05$) increased by 1.71 log CFU/mL from 1 to 2 h, and steadily increased ($p > 0.05$) by 0.27 log CFU/mL from 2 to 3 h. A sudden decrease of 0.71 log CFU/mL occurred at 4 h. Generally, a significant ($p < 0.05$)

growth increase (1.85 log CFU/mL) was observed for *S. cerevisiae* when subjected for 6 h at 2% bile (Figure 5).

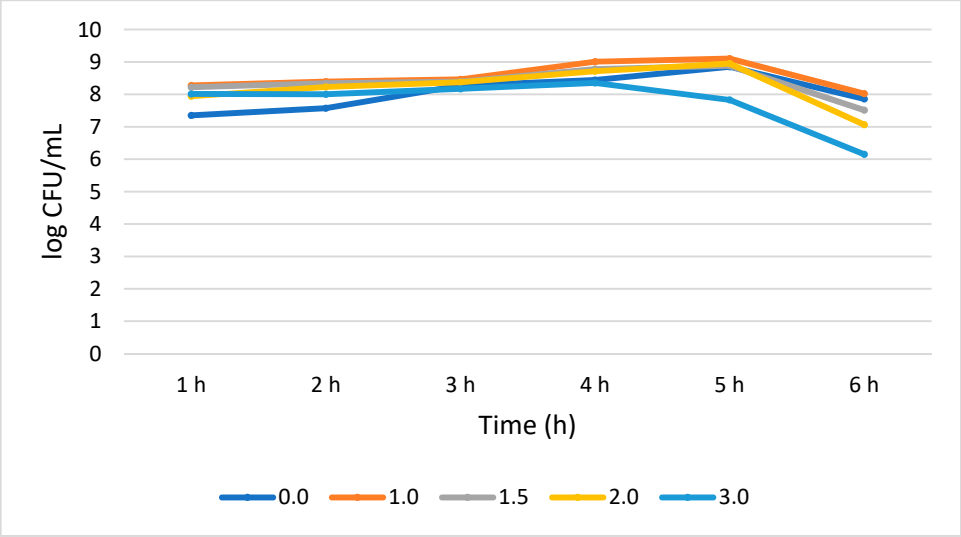

Figure 4. Bile tolerance of *L. plantarum* measured at different concentrations ranges from 0.0 (negative control) to 3.0%.

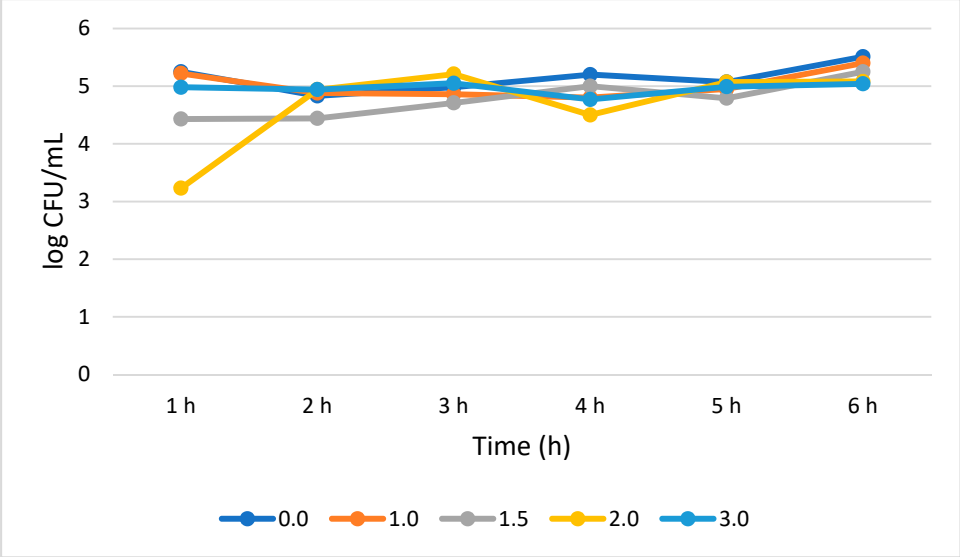

Figure 5. Bile tolerance of *S. cerevisiae* measured at different concentration ranges from 0.0 (negative control) to 3.0%.

B. longum tolerated and survived all the bile concentration. *S. cerevisiae* showed no statistically significant ($p > 0.05$) growth increase rates across the subjected 6 h period. *B. longum* demonstrated a 0.72, 0.36, 0.25, and 0.37 log CFU/mL growth increase at 1.0, 1.5, 2.0, and 3.0% bile concentrations, respectively, from 1 to 6 h. *B. longum* showed better survivability at 3.0% bile concentration as demonstrated by a total population of 9.43 log CFU/mL at 6 h. (Figure 6).

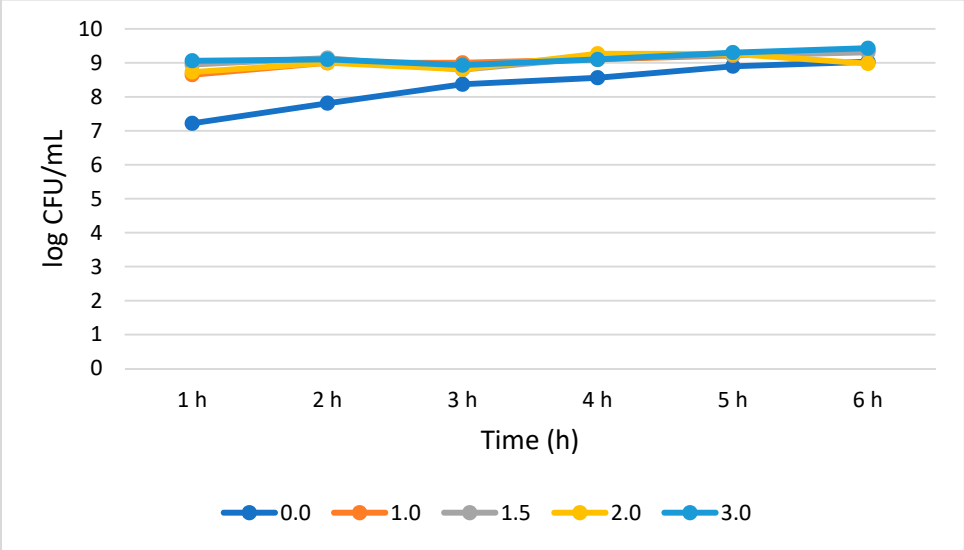

Figure 6. Bile tolerance of *B. longum* measured at different concentrations ranges from 0.0 (negative control) to 3.0%.

3.3. Antimicrobial Effect of L. plantarum, B. longum, and S. cerevisiae

The spot test displayed antimicrobial activity for the *L. plantarum* and *B. longum* results (Table 1). *L. plantarum* and *B. longum* showed an inhibitory effect for *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*. However, *S. cerevisiae* showed no inhibitory effect for all three pathogens. *L. plantarum* depicted the largest ZOI for all three pathogens compared to *B. longum*. The ZOI recorded against *E. coli* O157:H7 was 31.2 and 19.8 mm for *L. plantarum* and *B. longum*, respectively. Similarly, *L. plantarum* showed a 29.7 and 15 mm ZOI against *S. typhimurium* and *L. monocytogenes*, respectively. *B. longum* showed a 15.5 and 11.4 mm ZOI against *S. typhimurium* and *L. monocytogenes*, respectively. Both the *L. plantarum* and *B. longum* demonstrated a maximum antimicrobial activity against *E. coli* O157:H7, followed by, *S. typhimurium*, and *L. monocytogenes*.

Table 1. Antimicrobial activity of the probiotic strains determined by agar spot test.

	L. plantarum	***B. longum***	***S. cerevisae***
Tested pathogens	————-Zone of inhibition (mm)——		
E. coli: O157:H7	31.2 ^{a}	19.8 ^{b}	0 ^{c}
S. typhimurium	29.7 ^{a}	15.5 ^{b}	0 ^{c}
L. monocytogenes	15 ^{a}	11.4 ^{b}	0 ^{c}

^{a,b,c} Means within a row with no common superscript differ significantly ($p < 0.05$).

4. Discussion

Wang et al. [47], in agreement with our study, reported an increased *L. plantarum* cell viability with increased pH. According to their study, the survival rate of *L. plantarum* was 55.95% (18.80×10^7 cells/mL) and 18% (6.0×10^7 cells/mL) at pH 3.0 and 2.0, respectively. In this study, *L. plantarum* showed better survivability at a higher pH (3.5–6.0) than at a lower pH (2.5–3.0). At pH 3.5–6.0, *L. plantarum* survived after 5 h with an insignificant growth reduction of 0.06–0.44 log CFU/mL. On the other hand, at pH 2.5, the growth rate was significantly ($p < 0.05$) reduced by 4.32 log CFU/mL at 1 h. A similar study conducted by Anderson et al. [48] also reported a 6–7 log reduction of *L. plantarum* DSM 2648 and *Lactiplantibacillus rhamnosus* HN001 when challenged at pH 2.0 for 4 h, while no cell viability was observed at pH 4.0. However, in our study, no *L. plantarum* viable cells were detected after 2 h of incubation at pH 2.5. In contrast, Giri et al. [49] reported 5.8 log CFU/mL and 7.1 log CFU/mL viable

L. plantarum L7 strain at pH 2 and 3, respectively. There has been numerous studies that reported the survival of *L. plantarum* at pH 2.0–2.5 for 2–6 h [50–52].

S. cerevisiae showed tremendous tolerance when challenged to a broad range of pH, ranging from 2.5–6.0. Similarly to our current study, the ability of *S. cerevisiae* to survive in a broad range of pH (up to pH 10) was also reported by Khisti et al. [53]. To be an effective probiotic, *S. cerevisiae* should be capable of withstanding a low pH. Our study demonstrated the successful growth of *S. cerevisiae* at pH 2.5 with a total population of 5.70 log CFU/mL at 5 h. Several studies have also reported the survival of *S. cerevisiae* at a low pH, for example, Van der Aa Kühle et al. [54] and Pennacchia et al. [55] reported the survival of *S. cerevisiae* at pH 2.5 for 4 and 2.5 h (6.52–7.66 log CFU/mL), respectively. However, a decrease in *S. cerevisiae* cell counts was observed by Moradi et al. [56] at pH 1.5 and 2.0.

In this study, *B. longum* demonstrated a sensitivity to a lower pH (2.5 and 3.0), but also showed stability at a higher pH (3.5 and above). A previous study showed that after 1 h exposure at pH 2.5, the growth of *B. longum* had reduced by 3.75 log CFU/mL [57], while our study showed a 5.71 log CFU/mL growth reduction. Similarly, there have been several studies in which *B. longum* showed reduced growth at low pH. For example, Ashraf and Smith [58] reported 40% growth reduction at pH 3.0 for 3 h, Ding and Shah [59] reported a reduced population of 3.29 log CFU/mL at pH 2.0 after 2 h. However, a wide range of acid tolerance by *B. longum* was observed by Vernazza et al. [44]. Although many studies reported the sensitivity of *B. longum* at a lower pH, its acid tolerance could be improved by temporary acid stress as evidenced by many studies [51,60].

Our results display that *L. plantarum* survived different concentrations of bile ranging from 1.0–3.0% without a significant growth reduction (0.25–1.86 log CFU/mL). The findings of this study agree with the outcomes of several studies that showed *L. plantarum* could survive in a wide range of bile concentrations (0.05–4.0) from 3 h to >24 h with up to 100% survival rate [49,61]. Jiang et al. [51] reported a 1.17 log CFU/mL reduction of *L. plantarum* at 0.45% bile, while our study showed a 0.25 log CFU/mL reduction at 1.0% bile. However, several studies have reported a significant reduction in viable cells with increasing bile concentrations. For example, Wang et al. [47] reported a significant decrease from 19.50×10^7 to 10.88×10^7 CFU/mL of *L. plantarum* B1 with an increase in bile concentration from 0.1% to 0.5%. Similarly, the viability of the *L. plantarum* DSM2648 was reduced by 2 log units when the bile concentration increased from 0.5% to 1% [48].

In the current study, the viable population decreased from 5.51 to 5.04 log CFU/mL upon the increase in bile concentration from 1.0% to 3.0%. Similarly, Khisti et al. [53], Van der Aa Kühle et al. [54], and Agarwal et al. [62] reported the survivability of *S. cerevisiae* at 1.2, 0.3, and 0.9% bile concentrations, respectively. Meanwhile, some other researchers also reported the tolerance of *S. cerevisiae* in bile concentration ranging from 0.5 to 1.0% [42,63].

Bifidobacterium spp. are well known for their probiotic properties. Interestingly, this study showed an insignificant increase in *Bifidobacterium* growth ranging from 0.25 to 0.72 log CFU/mL when treated in 1.0–3.0% of bile for 6 h. Although we did not observe any significant growth reduction with increasing bile concentration, there have been several studies that reported a reduction of 30–80% when bile concentrations were increased from 0–3.0% [60,64]. Many researchers reported the tolerance of *B. longum* at varying concentrations of bile. Ashraf and Smith [58] reported a 1–2 log reduction of *B. longum* when treated in 2% bile salt for 12 h, while a sharp decrease of 5.4 log CFU/mL just after 5 min of exposure at 1% bile was observed in another study [57]. Supplementary materials are indicated in Table S1: Survival of L. plantarum, S. cerevisiae, and B. longum at different pH levels and Table S2: Survival of L. plantarum, S. cerevisiae, and B. longum at different bile concentrations.

Antimicrobial property is considered as an important characteristic of probiotics. A zone of inhibition (ZOI) with a diameter of 5 mm or larger was reflected as positive inhibition in our study. *L. plantarum* ZOI measured 15.0 mm, 31.2, and 29.7 for *L. monocytogenes*, *E. coli* O157: H7, and *S. typhimurium*, respectively. Indeed, previous studies have reported *L. plantarum*'s ability to inhibit *L. monocytogenes* and *E. coli* O157:H7 with a ZOI from 13.0 to 14.1 mm and from 10.0 to 16.5 mm, respectively [36,65]. A Polish study agrees with our study and reported a ZOI (18.27 ± 2.70 mm) for *L. monocytogenes* [38].

Similarly, there have been numerous studies which observed the positive inhibition by *L. plantarum* against significant pathogens [66,67]. It has been reported that many antimicrobial agents, for example, bacteriocins, organic acids, antimicrobial peptides, and hydrogen peroxide attribute the antimicrobial property of *L. plantarum* [27,68].

Numerous studies have shown that *B. longum* contains antagonistic property against major pathogens including both Gram-positive and Gram-negative bacteria [45]. Our study displayed the ZOI for *B. longum* in the range of 11.4–19.8 mm. *B. longum* has demonstrated strong inhibition against multidrug-resistant *E. coli* with a ZOI ranging from 11.77 to 23.10 mm [40] and against *Staphylococcus aureus*, *E. coli* O157: H7, and *L. monocytogenes* with ZOI of 11–17 mm [37]. However, Lahtinen et al. [41] reported 38 *Bifidobacterium* strains that showed no antimicrobial property against *E. coli* K-12 and *S. enterica* serovar Typhimurium ATCC 14028. It has been documented that bacteriocins or bacteriocin-like compounds produced by the members of the *Bifidobacterium* genus are well known for their antimicrobial properties against many pathogens [28,33,34].

In this study, *S. cerevisiae* showed no antagonistic effects (0 mm) against the tested pathogens. The inability of *S. cerevisiae* to inhibit the growth of *E. coli* O157:H7 and *S. typhimurium* as observed in the current study is in accordance with previous work by Srinivas et al. [42], where *S. cerevisiae* did not show any antagonistic activity towards *E. coli* O157:H7, *S. typhimurium* and *Salmonella paratyphi*. However, Khidhr and Zubaidy [69] reported a positive inhibition by *S. cerevisiae var. boulardii* against *S. enterica* with a 16 mm ZOI. Similarly, the antimicrobial property of *S. cerevisiae* against *S. aureus* and *L. monocytogenes* [39] and the reduction of the *Salmonella* load in chickens' caecum [70] has also been reported.

5. Conclusions

S. cerevisiae showed better acid and bile tolerance in vitro than *L. plantarum* and *B. longum*. However, *S. cerevisiae* did not show any antimicrobial activity against *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes*. *L. plantarum* and *B. longum* showed a strong inhibition against Gram-negative and Gram-positive bacteria. Our study suggests that *S. cerevisiae*, *L. plantarum*, and *B. longum* are potential probiotics which can be applied as an alternate for antibiotics in poultry production. Consumers' awareness of antimicrobial-resistant foodborne pathogen in poultry products has driven the demand for chicken raised without antibiotics (RWA). Hence, *L. plantarum*, *S. cerevisiae* and *B. longum* can be an alternative to antibiotics in the control of bacterial infections in poultry.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0472/10/9/368/s1, Table S1: Survival of *L. plantarum*, *S. cerevisiae*, and *B. longum* at different pH levels as determined by viable cell count, Table S2: Survival of *L. plantarum*, *S. cerevisiae*, and *B. longum* at different bile concentrations as determined by viable cell count.

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