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Evaluation of probiotic potential, anticancer, antioxidant, and cholesterol assimilation activity of *Weissella cibaria* strain isolated from fermented Idli batter

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Abstract
The strain *Weissella cibaria* MTCC 5948, isolated from fermented *idli* batter was evaluated for its probiotic potential based on its acid and bile tolerance, auto-aggregation, co-aggregation, antimicrobial activity, and bile salt hydrolase activity. Additionally, its biofunctional attributes, including antioxidant activity, anticancer potential, and cholesterol assimilation, were assessed. The strain demonstrated a viability of 3.92 log CFU/mL after 3 h at pH 2.0 and 5.23 log CFU/mL at 1% bile salt after 4 h of incubation. Further, the strain showed an auto-aggregation of 35.49% and the highest co-aggregation of 34.18% with *Escherichia coli*. It exhibited notable antimicrobial activity, particularly against *S. typhimurium*, with an inhibition zone of 11.66 mm. Furthermore, *W. cibaria* MTCC 5948 showed antioxidant activity of 28% and higher nitrite release compared to the control strain *Lactobacillus rhamnosus* GG, indicating potential anticarcinogenic effects. Its cholesterol assimilation of 53.11% highlights its potential in managing hypercholesterolemia. These results suggest that *W. cibaria* MTCC 5948 holds promise as a probiotic.

Keywords: *Weissella cibaria* MTCC 5948, lactic acid bacteria, food, health, bile salt hydrolase activity

Introduction
Weissella is a genus of lactic acid bacteria (LAB) commonly found in fermented foods, widely recognized for its potential probiotic properties. *Weissella* species are Gram-positive, non-spore-forming, catalase-negative, and obligate heterofermentative lactic acid bacteria. These bacteria exhibit functional and technological properties that enhance the safety, nutritional value, and sensory qualities of foods. Among them, *Weissella cibaria* and *Weissella confusa* are recognized for their high production of exopolysaccharides and oligosaccharides, contributing to their texturizing capabilities. Additionally, several *Weissella* species isolated from food and animal sources are reported to offer health benefits, such as cholesterol reduction, antioxidant activity, immunomodulation, oral health improvement, and anticancer effects (Unnikrishnan and Sreeja, 2021) [1].

Although many *Weissella* strains are considered promising probiotics due to their biotechnological applications, some strains exhibit opportunistic pathogenic behaviour, which limits their universal applicability in food products (Liu *et al*., 2024) [2]. Probiotic strains must remain viable and reach the colon in their live form to confer health benefits (Elvarsi *et al*., 2015). To meet this criterion, they need to survive the harsh conditions of the gastrointestinal tract. *In vitro* tests are commonly used to evaluate the probiotic potential of these strains, including their tolerance to gastric pH and bile salts, adhesion properties, antimicrobial activity against pathogens, and bile salt hydrolase activity. Gut-colonizing bacteria provide numerous health benefits, and research on LAB has highlighted their bio-functional properties such as antimicrobial, antioxidant, antidiarrheal, antiviral, immunomodulatory, hypocholesterolemic, antidiabetic, and anticancer activities (Zhang *et al*., 2025) [4].

Weissella strains produce a variety of antagonistic substances, including organic acids and bacteriocins. Yu *et al*. (2019) [5] found that the antimicrobial activity of *W. cibaria* JW15 was stronger than that of the probiotic strain *Lactobacillus rhamnosus* GG (LGG).

The *Weissella* strain exhibited greater inhibition against *Listeria monocytogenes* and *Salmonella enteritidis* and produced higher levels of lactic acid and acetic acid compared to LGG. In a separate study, Huy *et al*. (2020) [6] reported that *W. cibaria* HN05, isolated from white pacific shrimp, displayed strong antagonistic activity against *Vibrio parahaemolyticus*, with antagonistic activity ranging from 220 AU/ml to 500 AU/ml. Additionally, *W. cibaria* HN05 inhibited *E. coli* growth, demonstrating an antagonistic activity of 240 AU/mL.

The cell-free extracts (CFS) and exopolysaccharides (EPS) from various *Weissella* strains isolated from fermented foods exhibit significant antioxidant activity. These strains produce several antioxidant enzymes, including superoxide dismutase, glutathione reductase, and NADH oxidase (Unnikrishnan and Sreeja, 2021) [1]. Yu *et al*. (2019) [5] investigated the antioxidant properties of *W. cibaria* JW15 and found that intact JW15 cells demonstrated a radical scavenging effect ranging from 23.53% to 60.85%, along with a lipid peroxidation inhibition of 33.31% in the tested assays. Similarly, Amrutha *et al*. (2019) [7] evaluated the antioxidant activity of *Weissella* strains isolated from tender coconut water and reported that the EPS produced by *W. cibaria* DMA 18 exhibited notable antioxidant activity. The DPPH assay revealed antioxidant activity levels of 27% and 33.55% with sample volumes as low as 10 µl and 6 µl, respectively. However, research on the cholesterol assimilation ability of *Weissella* strains, particularly *Weissella cibaria*, remains limited.

Nitrosamines, carcinogens formed by the reaction of nitrites with amines in food or the stomach, pose significant health risks. Some bacteria can degrade nitrosamines or convert them into harmless substances like carbon dioxide (Li *et al*., 2024) [8]. Studies on *Weissella* have explored the degradation of diphenylnitrosamine (DPN) to evaluate their anti-carcinogenic properties, with nitrite release primarily influenced by probiotic enzymes or gut microflora. The present study focuses on evaluating the probiotic potential, anticancer, antioxidant, and cholesterol assimilation activity of a *Weissella cibaria* MTCC 5948 isolated from fermented idli batter.

Material and methods

Weissella cibaria MTCC 5948 was isolated from fermented idli batter in the Dairy Microbiology Department at SMC College of Dairy Science, Kamdhenu University, Anand. It was cultured in MRS medium by incubating at 37°C for 24 hours and subsequently stored at 5°C. Sub-culturing was performed every 10 days throughout the study. Other pathogenic bacterial strains used in the study included *Escherichia coli* MTCC 1687, *Listeria monocytogenes* MTCC 657, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* MTCC 737, and *Bacillus cereus* MTCC 1272. These strains were grown in nutrient broth by incubating at 37°C for 24 hours and stored at 5°C. Moreover, *Lactobacillus rhamnosus* GG ATCC 53103, purchased from Himedia (Mumbai, India), was also included in the study as control strain and maintained in MRS broth.

Tolerance to low pH and bile

The tolerance of *Weissella* strain to low pH was evaluated by the method given by Kathiriya *et al*. (2015) [9]. MRS broth was adjusted to pH levels of 1.0, 2.0, 3.0, and 6.5 (control) and inoculated with 2% active strain. Samples were collected at intervals (0, 1, 2, and 3 hours), diluted, plated on MRS agar, and incubated at 37°C for 48–72 hours. Viable cell counts were determined and expressed as log CFU/mL.

The bile tolerance of *Weissella* strain was evaluated by the method given by Zoumpopoulou *et al*. (2008) [10] with some modifications. Cultures were activated in MRS broth, centrifuged, washed with PBS, and resuspended. These were added to MRS broth containing varying bile salt concentrations (0.3%, 0.5%, 1%) and to a control without bile salt, then incubated at 37°C. Samples were collected at intervals (0, 1, 2, 4 hours), diluted, plated on MRS agar, and incubated to determine viable cell counts, expressed as log CFU/mL.

Auto-aggregation and co-aggregation

The auto-aggregation and co-aggregation of the *Weissella* strain were assessed using the modified method described by Anandhraj *et al*. (2015). Following cell harvesting and washing, the cells were resuspended in PBS and incubated at 37°C for 2 and 5 hours. The absorbance at 600 nm was then measured to determine the auto-aggregation percentage.

$$\text{Auto aggregation \%} = \frac{(1 - \text{A (Upper suspension)}}{\text{A (Total bacterial suspension)}} \times 100$$

Were,

A Upper suspension=OD at 600 nm of upper suspension after 2h / 5h

A Total suspension=OD at 600 nm of Total bacteria suspension after 2h / 5h

To assess the co-aggregation capability, 10 mL of culture was collected, and cells were harvested by centrifugation at 5,000 rpm for 10 minutes. Equal volumes (2 mL each) of *Weissella* strain and pathogenic bacterial strains were combined and incubated at 37°C for 5 hours. The optical density of the mixed cultures was measured at 600 nm. 2ml of either *Weissella* suspension or pathogen suspension was used as control. Co-aggregation of *Weissella* strain was tested against *E. coli* MTCC 1687, *S. typhimurium* ATCC 14028, *L. monocytogenes* MTCC 657, and *S. aureus* MTCC 737. The co-aggregation percentage was calculated using the formula

$$\text{Co-aggregation} = \frac{(\text{A testbacteria} + \text{A } Weissella) - 2(\text{A mixed strain})}{(\text{A testbacteria} + \text{A } Weissella)} \times 100$$

Were,

A Test bacteria -OD 600 nm Pathogenic Bacteria

A *Weissella* -OD 600 nm *Weissealla*

A mixed strain -OD 600 nm of *Weissella* + Pathogen

Antimicrobial activity

The antimicrobial activity of *Weissella* strain was evaluated using the agar well diffusion method. The indicator pathogens, including *E. coli* MTCC 1687, *S. typhimurium* ATCC 14028, *L. monocytogenes* MTCC 657, *S. aureus* MTCC 737, and *B. cereus* MTCC 1272 were cultured and used in the assay. The cell-free supernatant (CFS) of the *Weissella* strain was collected after centrifugation and tested for antimicrobial effects. The indicator pathogens were mixed with nutrient agar, and wells were created in the solidified agar, into which the CFS was placed. A 3% citric acid solution served as the positive control. After incubation, the antimicrobial activity was measured as the inhibition zone around the wells.

Bile salt hydrolase (BSH) activity
This test was conducted following the method outlined by Lee *et al*. (2011) [11]. A direct plate assay was used to detect BSH activity. The active isolates were streaked onto solidified MRS agar containing 0.5% (w/v) bile (sodium taurodeoxycholate hydrate, Himedia, Mumbai, India) and 0.37 g/L of $CaCl_2$ (Loba-chem, Mumbai, India). The Petri dishes were incubated anaerobically at 37°C for 48 hours in an anaerobic jar. Activity was indicated by the formation of a distinct precipitate around the colonies after incubation.

Antioxidant activity
The DPPH radical scavenging activity of *Weissella* strain was evaluated using the method described by Yu *et al*. (2019) [5]. To assess DPPH activity, 200 µl of *Weissella* suspension or PBS were mixed with 2 ml of DPPH solution (Himedia, Mumbai, India) and incubated in the dark at room temperature for 20 minutes. The absorbance of each sample was then measured at 517 nm.

$$\% \text{ Radical scavenging activity} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

A Control-Absorbance of PBS at 517 nm
A Test-Absorbance of Bacterial suspension at 517 nm

Anti-carcinogenic activity
The anticancer potential of the *Weissella* strain was evaluated using a nitrosamine degradation assay. The ability of *Weissella* strains to degrade diphenylnitrosamine (DPN) was assessed following the method described by Duangjitcharoen *et al*. (2014) [12].

Preparation of standard curve
A standard curve was created using a standard nitrite solution. Sulfanilamide solution was prepared by dissolving 1 g of sulfanilamide (Sigma-Aldrich, Bengaluru, India) in 500 ml of dilute hydrochloric acid. The coupling reagent was prepared by dissolving 0.2 g of N-(1-naphthyl)-ethylenediamine dihydrochloride (Sigma-Aldrich, Bengaluru, India) in 100 ml of distilled water. Sodium nitrite (Himedia, Mumbai, India) solutions of varying concentrations were prepared in double-distilled water using a 100 ml volumetric flask. To each flask, 10 ml of sulfanilamide solution was added and mixed thoroughly. After 3 minutes, 1 ml of the coupling reagent was added, and the solution was diluted to the 100 ml mark with distilled water. The mixture was left for 15 minutes. Absorbance was measured at 540 nm using a Systronic PC-based double-beam spectrophotometer. The standard curve was plotted, showing absorbance as a function of nitrite concentration.
To prepare the bacterial suspension, *Weissella* strains were cultured in MRS broth until reaching the mid-exponential phase, then centrifuged at 10,000 rpm for 10 minutes at 4°C. The resulting cells were resuspended in 0.2 M potassium phosphate buffer (pH 7.0) to achieve a final concentration of approximately 2×10^9 viable cells per millilitre.

Assay
The bacterial suspensions (1 ml) were incubated at 37°C for 20 hours in 50 ml Erlenmeyer flasks containing 0.1 ml of DPN (Sigma-Aldrich, Bengaluru, India), prepared as solutions of 1, 50, and 100 µmol/ml in acetone (Loba Cheme, Mumbai). Following incubation, 10 ml of sulphanilamide solution (Sigma-Aldrich, Beng, India) was added to each flask along with 1 ml of the coupling reagent. The mixture was diluted to the mark with distilled water and left to stand for 15 minutes. The absorbance of the solutions was then measured at 540 nm using a spectrophotometer (Systronic PC-based double-beam spectrophotometer 2206, Ahmedabad, India). This analysis quantified the amount of nitrite released in the mixture by *Weissella*.

Cholesterol assimilation assay
The cholesterol assimilation by the *Weissella* strain was evaluated using the method described by Anandharaj *et al*. (2015) [13]. *Weissella cibaria* MTCC 5948 was incubated in MRS broth with bile salts, sodium thioglycolate, and cholesterol for 24 hours. After centrifugation, the supernatant was treated with ethanol, KOH, and hexane, followed by phase separation. The hexane layer was collected, and the cholesterol content was determined using a standard curve after evaporating the hexane.

Statistical analysis
The data are presented as mean ± standard deviation and were statistically analyzed using a Factorial Completely Randomized Design (Steel and Torrie, 1980) where applicable. Differences were considered significant at $p<0.05$.

Results and discussions
Potential probiotic strains must withstand acidic environments and bile secretions to successfully navigate through the stomach and small intestine. The pH of intestinal gastric juice typically ranges between 2.0 and 3.0, which destroys most ingested microorganisms. Bile also plays a crucial role in the gut's defence mechanisms, making bile salt resistance a key characteristic of potential probiotics (Wang *et al*., 2020) [14]. Gastric digestion can last up to three hours when the gastric juice pH is approximately 3.0 (Liu *et al*., 2024) [2]. Studies indicate that the physiological concentration of human bile ranges from 0.3% to 0.5%, with food transit through the small intestine taking about four hours. During this time, the average bile concentration is around 0.3%. The *Weissella cibaria* MTCC 5948 strain was evaluated for its ability to grow in low-pH conditions. In this assessment, the strain's survivability was tested using MRS broth adjusted to pH levels of 1, 2, and 3 with 1N HCl, while pH 6.5 served as the control (Figure 1).
The results (Figure 1) indicate that the strain was able to survive under low pH conditions. *Weissella cibaria* MTCC 5948 exhibited the highest viability at pH 3 and maintained its viability (7.83 log CFU/mL) for up to 3 hours of exposure. Although the strain survived at pH 2, a gradual reduction in viability was observed with increasing exposure time (3.92 log CFU/mL after 3 h). At pH 1, the organism lost its viability after 1 hour of incubation.
The viability of *Weissella cibaria* MTCC 5948 when exposed to various concentrations of bile is given in figure 2. The strain exhibited the highest viability (7.18 log CFU/mL) at 0.3% bile concentration after 4 hours of exposure. However, at a 0.5% concentration, a gradual reduction in cell count was observed from 1 hour to 4 hours of exposure. The lowest viability (5.23 log CFU/mL) was recorded at 1.0% bile concentration after 4 h of incubation.
For probiotics to be effective, they must survive the stomach's acidic conditions, the colon's alkaline environment, and the presence of bile salts in the small intestine. Amrutha *et al*.

(2019) [7] revealed that the *W. cibaria* strain DM18, sourced from tender coconut water, was capable of withstanding pH 3.0 and a bile salt concentration of 0.6% (w/v) for up to 3 hours. Similarly, Xiong *et al*. (2018) [16] analysed the probiotic properties of the *W. confusa* strain from a giant panda and discovered that the *W. confusa* BSP201703 strain could endure pH 2.0 and 0.3% (w/v) bile salt for 2 hours. These observations are consistent with findings related to *W. cibaria* MTCC 5948. Furthermore, Liu *et al*. (2024) [2] documented a survival rate of 72% for *W. cibaria* P-8 after 3 hours of exposure to pH 2.0 and 71.56% under 0.3% bile salt conditions. Altogether, these results confirm that *W. cibaria* MTCC 5948 can thrive in low-pH and 0.3% bile salt environments, demonstrating its resilience to gastrointestinal conditions.

Bacterial aggregation, occurring either within the same strain (auto-aggregation) or between different species and strains (co-aggregation), is a key factor in the human gut, where probiotics must demonstrate efficacy. Auto-aggregation is essential for probiotic strains to adhere to the oral cavity, gastrointestinal tract, and urogenital tract, while co-aggregation contributes to forming a protective barrier that hinders pathogen colonization (Kiran *et al*, 2025) [17]. The auto-aggregation activity of *Weissella cibaria* MTCC 5948 was assessed after 2h and 5 hours of incubation, and the strain demonstrated an increase in auto-aggregation percentage over this period (Table 1).

The *Weissella cibaria* strain showed moderate co-aggregation with all tested pathogen strains. The highest co-aggregation was observed with *E. coli* MTCC 1687 (34.18%), while the lowest was with *L. monocytogenes* MTCC 657 (30.04%) (Table 2). This aggregation activity of the *W. cibaria* strain indicates its adhesion potential. Pabri *et al*. (2020) [18] examined the auto-aggregation ability of *W. paramesenterioides* isolates from fruits, reporting that five *W. paramesenterioides* isolates exhibited auto-aggregation levels ranging from 22% to 36% after 4 hours of incubation, which aligns with the results obtained for our strain *Weissella cibaria* MTCC 5948. Cirat *et al*. (2024) [19] found that the co-aggregation of *W. cibaria* VR81 with *L. innocua* was 21.0% after 4 hours of incubation. The adhesion ability of lactic acid bacteria (LAB) is strain-specific, which underscores their probiotic potential. The health benefits of probiotics are linked to the composition of the gut microbiota and their ability to adhere to epithelial and mucosal surfaces (Kim *et al*., 2022) [20].

Lactic acid bacteria exhibit antagonistic activity against various bacteria, including food spoilage and food-borne pathogens. Several mechanisms contribute to this inhibition, such as the production of organic acids, diacetyl, hydrogen peroxide, and/or bacteriocins (Mudgal, 2015) [21]. The antimicrobial activity of *W. cibaria* MTCC 5948 against different pathogens is shown in Table 3. Well-agar diffusion method was employed in the study, with a well diameter of 6 mm. The organism exhibited the largest zone of inhibition (11.66 mm) against *S. typhimurium* ATCC 14028 and the smallest (7.33 mm) against *S. aureus* MTCC 737. The strain demonstrated moderate antimicrobial activity against the tested pathogens. Wang *et al*. (2020) [14] reported the antimicrobial effects of *W. confusa* YM5S1 against *E. coli*, while *W. confusa* YM5S2 showed a strong inhibitory effect on *S. aureus* and both strains were inhibitory to *Salmonella typhimurium*.

Bile salt hydrolase (BSH) activity is essential for bacterial growth and intestinal colonization, as it deconjugates bile salts that are typically excreted in the gastrointestinal tract. The BSH enzyme catalyzes the hydrolysis of conjugated bile acids, resulting in the release of deconjugated bile acids and amino acids. *W. cibaria* MTCC 5948 exhibited significant BSH activity, as evidenced by the formation of a distinctive precipitate around the colonies (Figure 3) after 48 hours of incubation. Nami *et al*. (2024) [15] reported that the *W. confusa* ABRIIFBI-96 isolate also displayed strong bile salt hydrolase activity with both glycolic and taurocholic bile salts, correlating with the BSH activity observed in our *W. cibaria* strain.

Weissella strains exhibit resistance to reactive oxygen species (ROS)-induced oxidative stress by expressing antioxidant enzymes such as superoxide dismutase, glutathione reductase, and NADH peroxidase (Yu *et al*., 2019) [5]. The antioxidant activity of intact *Weissella* cells was evaluated by measuring their free radical scavenging activity. The percentage of free radical scavenging activity was assessed using DPPH radical scavenging, with 1 mg/ml ascorbic acid in PBS serving as a positive control. *W. cibaria* MTCC 5948 demonstrated a promising radical scavenging activity of 28.48% compared to 53.76% of positive control ascorbic acid (Table 4). Yu *et al*. (2019) [5] also reported the antioxidant activity of *W. cibaria* JW15. They reported that the intact cells of *W. cibaria* JW15 exhibited a radical scavenging effect ranging from 23.53% to 60.85%.

Nitrosamines, a group of strong carcinogens, pose significant health risks to humans. They are formed when nitrites react with secondary amines in food, either naturally or through bacterial activity, and can be present in food at concentrations up to 10 g/kg. In the human stomach, nitrosamines can also form when nitrate and nitrite interact with amines and amides. Some bacteria can break down nitrosamines into their original amine and nitrite forms, while others can oxidize dimethylamine to carbon dioxide (Li *et al*., 2024) [8]. The degradation of nitrosamine from diphenylnitrosamine (DPN) was evaluated to assess the anti-carcinogenic properties of the *Weissella* strain. The amount of nitrite released in the mixture was calculated with the help of standard curve. The standard curve was prepared by using sodium nitrite in the range of 0.1 to 4.0 µmol/ml and spectrophotometric reading was observed and the amount of nitrate released by *W.cibaria* MTCC 5948 is given in Table 5. The amount of nitrite released from DPN may be influenced by the concentration of available DPN, but it is primarily determined by the enzymes produced by the probiotic cultures or other microflora present in the gut.

It was observed that the release of nitrite was significantly higher in *W. cibaria* MTCC 5948 when reacted with 100 µg of DPN. The release of nitrite was significantly higher from 100 µg of DPN (2.48 µmol/ml) compared to 1µg (0.33µmol/ml) and 50 µg (2.28µmol/ml). *L. rhamnosus* GG showed lesser release of nitrate from DPN (0.38,2.08 and 2.20 µmol/ml from 1,50 and 50µg of DPN respectively). It was observed that amount of nitrite released from DPN in terms of percentage breakdown was maximum at the concentration of 1µg. This increase obviously at 50 and 100 µg, but the increase was not in proportion to the concentration of DPN.

Probiotic bacteria are believed to exert a hypocholesterolemic effect through various mechanisms, including cholesterol assimilation, binding of cholesterol to cellular surfaces, cholesterol co-precipitation, interference with micelle formation for intestinal absorption, and bile acids deconjugation through the secretion of bile salt hydrolase (Anandhraj *et al.*, 2015). Cholesterol assimilation of *Weissella cibaria* MTCC 5948 was found to be 53.11 ± 4.85%, which is very promising. Dubey *et al.* (2015) [22] investigated the cholesterol assimilation activity of *W. confusa* strains isolated from uttapam batter. They found that *W. confusa* AJ53, AJ67, and AJ79 demonstrated cholesterol assimilation of 60%, 59.5%, and 57%, respectively. These findings align with the cholesterol assimilation percentage of *Weissella cibaria* MTCC 5948. Additionally, Liu *et al.* (2024) [2] reported that the *W. viridescens* strain 6996 exhibited a cholesterol assimilation of 25.53%.

Table 1: Auto- aggregation (%) of *W. cibaria* MTCC 5948 at different time intervals

Auto-aggregation (%)	
2 hours	**5 hours**
17.17±0.21	35.49 ±0.34

Table 2: Co- aggregation (%) of *W. cibaria* MTCC 5948 strain against various pathogens (2h of incubation)

Co- aggregation (%)			
***Escherichia coli* MTCC 1687**	***Listeria monocytogenes* MTCC 657**	***Salmonella typhimurium* ATCC 14028**	***Staphylococcus aureus* MTCC 737**
34.59±0.44	28.22±0.52	31.5±1.01	31.22±0.44

Table 3: Antimicrobial activity (Inhibition Zone in mm) of *W. cibaria* MTCC5948 against pathogens

Zone of Inhibition in (mm)				
***E.coli* MTCC 1687**	***L. monocytogenes* MTCC 657**	***S. typhimurium* ATCC 14028**	***B. cereus* MTCC 1272**	***S. aureus* MTCC 737**
10.66±1.15	10.00±1.01	11.66±1.52	10.33±250	7.33±2.30

Table 4: Anti-oxidative Activity of *W. cibaria* MTCC 5948 by DPPH Method

Sample	Radical scavenging activity (%)
W. cibaria MTCC 5948	28.48 ± 0.33
Ascorbic acid	53.76±0.33

Table 5: Anti-carcinogenic activity of *W.cibaria* MTCC 5948 by nitrosamine degradation assay

Organism	Amount of nitrite released from DPN (μmol/mL)		
	1 μg	**50 μg**	**100 μg**
W. cibaria MTCC 5948	0.33 ± 0.03	2.28 ±0.1	2.48 ±0.12
L. rhamnosus (GG) ATCC 53103	0.38± 0.01	2.08 ±0.01	2.20 ± 0.04

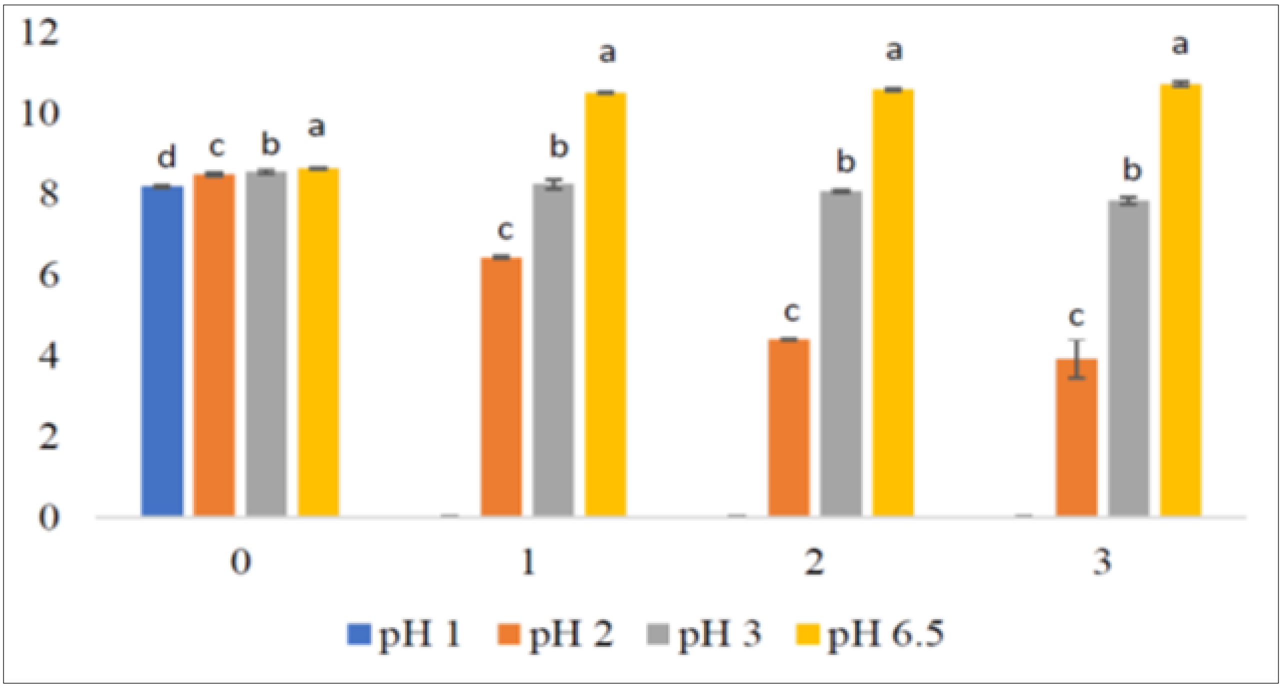

Fig 1: Viability (log CFU/mL) of *W. cibaria* MTCC5948 after exposure to different pH at specific intervals. Different superscripts in the bars denote significant differences (p< 0.05) between the pH at a specific incubation hour. Each observation is a mean ± SD of three replicates (n=4). Viability could not be observed in pH 1.0 after I h of incubation.

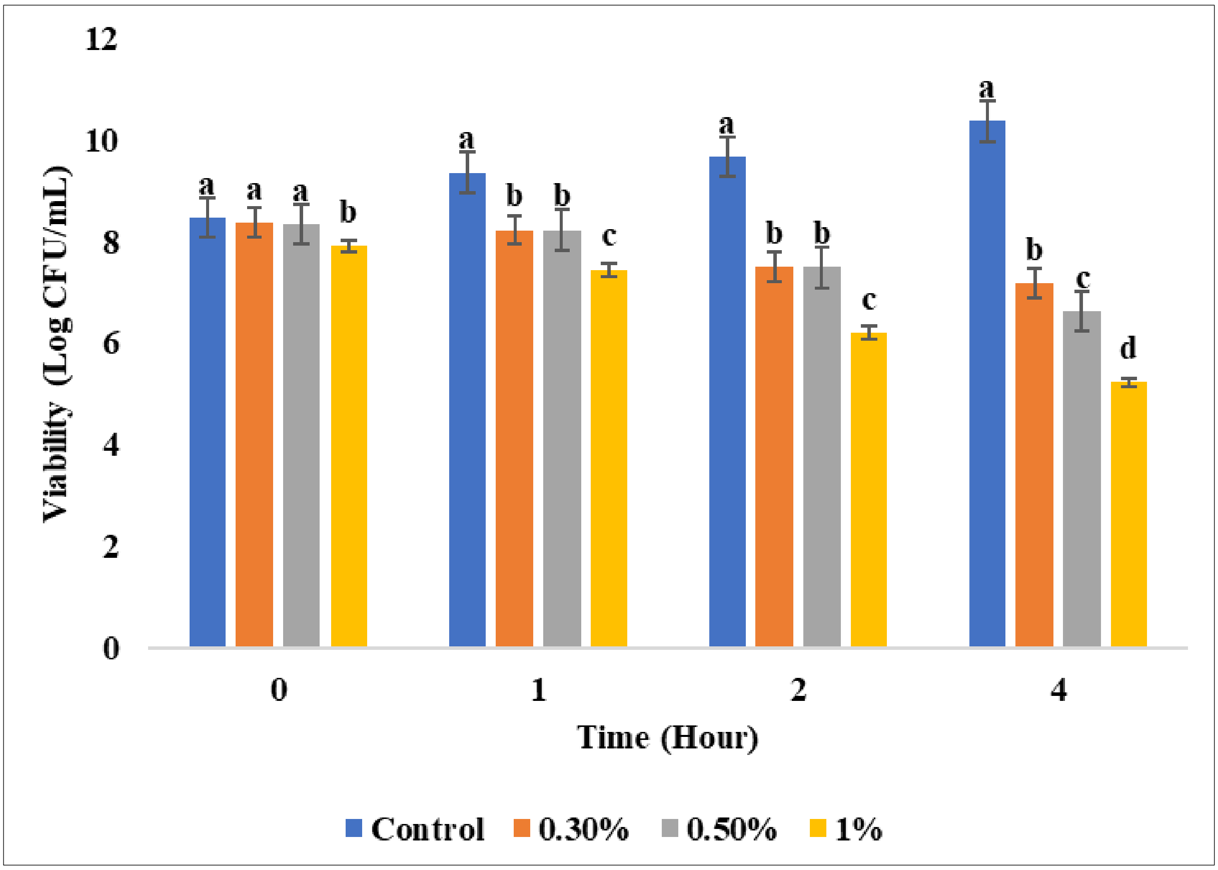

Fig 2: Viability (log CFU/mL) of *W. cibaria* MTCC 5948 after exposure to different bile salt concentrations at specific intervals. Each observation is a mean ± SD of three replicates (n=4). Different superscripts in the bars denote significant differences (*p*<0.05) in the viability at different bile concentrations at a specific incubation hour.

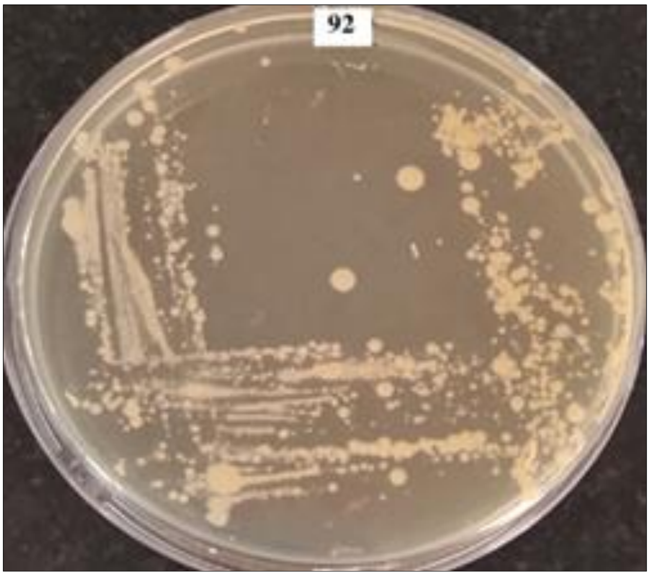

Fig 3: Bile salt hydrolase activity of *W.cibaria* MTCC 5948

Conclusion
The findings of this study demonstrate that *Weissella cibaria* MTCC 5948 possesses significant probiotic potential, characterized by its tolerance to acidic and bile conditions, moderate auto-aggregation, strong co-aggregation, and notable antimicrobial activity against pathogenic microorganisms. Its ability to exhibit bile salt hydrolase activity, antioxidant properties, anticarcinogenic potential, and cholesterol assimilation further underscores its multifunctional health benefits. These attributes make *W. cibaria* MTCC 5948 a promising candidate for use as a probiotic in functional foods, contributing to improved human health and the development of novel therapeutic applications.

Future Aspects
Future study can focus on investigating the molecular mechanisms underlying the bile salt hydrolase activity, cholesterol assimilation, and radical scavenging properties of *Weissella cibaria* MTCC 5948 to better understand its health-promoting actions.

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