Applied Biological Research 24(3): 288-297; (2022)
DOI: 10.5958/0974-4517.2022.00035.0

In vitro EVALUATION OF ANTIDIABETIC, ANTIOXIDANT AND PROBIOTIC ATTRIBUTES OF *Lactobacillus casei* strain NCDC 357

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(Received 22 February, 2022; accepted 12 June, 2022)

ABSTRACT

The present study was aimed to evaluate the antidiabetic and antioxidant activities of a lactic acid bacterial strain '*Lactobacillus casei* NCDC 357', and to investigate its probiotic characteristics. The antidiabetic activity was examined by evaluating the α-glucosidase and α-amylase inhibitory activities. *L. casei* strain NCDC 357 exhibited antidiabetic activities by α-glucosidase (67.7% and IC_{50} 38.08 µL) and α-amylase (53.6% and IC_{50} 49.96 µL). The strain showed antioxidant activities by DPPH (53.4% and IC_{50} 77.60 µL) and ABTS (48.7% and IC_{50} 51.48 µL). Further, the strain was resistant to simulated gastrointestinal juice and showed potential for health promotion based on adhesion, antibiotic susceptibility, and anti-microbial activities. *L. casei* NCDC 357 appeared to be a potential probiotic having beneficial health effects.

Keywords: Antidiabetic activity, antioxidant activity, *Lactobacillus casei*, probiotic, strain evaluation

INTRODUCTION

The World Health Organization has described probiotics as living microorganisms which provide health benefits to the host when consumed in suitable quantities (FAO/WHO, 2002). Probiotics may be a single culture or a mixed-culture of live microorganisms which are known to either alleviate the lactose intolerance, peptic ulcers, and diarrhea or depict antifungal, anticancer and anti-allergic properties. Probiotic bacteria generally belong to lactic acid bacteria (LAB), *Bifidobacterium, Saccharomyces*, *Streptococcus* and enteric. LAB, generally regarded as safe (GRAS), are broadly used in functional food development with probiotic properties (Saharan *et al*., 1998; Wegh *et al*., 2019). LAB are found in the intestines of humans and animals and naturally fermented foods. During microbial metabolization of carbohydrates various short chain fatty acids are produced, which serve as energy source for intestinal epithelial cells, help in metabolic regulation and strengthen the immune system (Sharma and Saharan, 2014; Ashwin *et al*., 2018). Due to gut health and beneficial health properties the demand for probiotics is rapidly increasing.

Reactive oxygen species (ROS), which include free radicals like superoxide radicals, hydroxyl radicals, and non-free radicals like H_2O_2 and singlet oxygen, as well as numerous forms of active oxygen, are involved in a number of physicochemical reactions in body and aging. Derivatives of oxygen, referred to as ROS, are constantly generated inside human body. The antioxidants in body detoxify these ROS. Excessive ROS production or insufficient antioxidant defenses, on the other hand, readily cause oxidative damage to the biomolecular components, such as lipoproteins, proteins, lipids, and DNA. Thus, oxidative damage is linked to many chronic

human diseases, such as diabetes, cancer, atherosclerosis, arthritis, neurological disorders, and aging (Phaniendra *et al.*, 2015). Antioxidants are vital substances that have the ability to protect body from the damages caused by free radical-induced oxidative stress (Won *et al*., 2021). Radicals and other ROS, formed constantly in human body, are removed by enzymatic and non-enzyme antioxidant defense systems. Studies have demonstrated the antioxidative characteristics of LAB (Sharma and Saharan, 2016; Chaudhary and Saharan, 2019; Cizeikiene and Jagelaviciute, 2021).

Diabetes mellitus (DM) is a chronic metabolic condition caused by insufficient insulin synthesis or action. Due to the lowered insulin secretion and activity, glucose balance is disturbed in diabetes patients. Genetic factors, overweight, westernized food, and a lack of physical activity are among the various causes of type 2 diabetes mellitus (T2DM), the most frequent type of diabetes (Punthakee *et al*., 2018). T2DM is generally treated with medicines; however, changing the gut microbial diversity by probiotics might be a critical factor in preventing obesity and diabetes (Gurung *et al*., 2020). Recent studies have revealed that probiotics attenuate diabetic symptoms by improving the gut microbiota composition, enhancing insulin sensitivity, and reducing autoimmune reactions (Sharma *et al.*, 2014; Tabrizi *et al*., 2018). Beneficial gut flora also reportedly decrease blood glucose levels by triggering the release of enzymes and hormones (Gerard and Vidal, 2019).

Glucose, produced as a result of degradation of oligosaccharides and disaccharides by glucosidase enzymes expressed at the intestinal brush border is absorbed in the intestine. Activity and concentration of intestinal glucosidase determine the level of postprandial glucose (Choi *et al*., 2017; Chaudhary and Saharan, 2018). The inhibition of α-glucosidase, which catalyzes the polysaccharide breakdown process, lowers the level of postprandial glucose by prolonging the glucose secretion and absorption, preventing the glucose production after a meal, carbohydrate metabolism, and excess glucose assimilation. As a result, the inhibition of carbohydrate hydrolases such as α-glucosidase, is a suitable approach for reducing postprandial hyperglycemia in T2DM patients (Kumar *et al*., 2011). Various commercially available α-glucosidase targeted-inhibitors including acarbose, voglibose, and miglitol bind to enzymes and impede their function. However, these inhibitors may cause gastrointestinal disorders such as abdominal cramping and diarrhea, which have limited their use in medicinal practices (Dileep *et al*., 2018). In present study, the probiotic, antidiabetic and antioxidant attributes of *Lactobacillus casei* NCDC 357 were explored.

MATERIALS AND METHODS

The probiotic *Lactobacillus casei* strain NCDC 357, used in present study, was procured from the National Dairy Research Institute (NDRI), Karnal, Haryana, India.

Evaluation of probiotic properties of L. casei NCDC 357

Bile, acid, and phenol tolerance: The bile salt tolerance test was assessed as per the method of Kiani *et al.* (2021a). Briefly, *L. casei* NCDC 357 was grown in 9 mL of fresh MRS (de Man, Rogosa and Sharpe) broth with 0.2, 0.4 and 0.6% (w/v) bile oxgall salt (Sigma Aldrich, USA) and incubated at 37°C for 4 h. The growth rate was measured spectrophotometrically (Systronics, India) at 600 nm.

The acid resistance test of *L. casei* NCDC 357 was carried out as per the method of Kiani *et al.* (2021b) with slight modifications. Briefly, 1 mL freshly grown *L. casei* NCDC 357 was added to 9 mL MRS broth at various pH (2, 4 and 6), and incubated at 37°C for 3 h. Then optical density (OD) was measured at 600 nm using a spectrophotometer (Systronics, India).

To enumerate the phenol tolerance, *L. casei* NCDC 357 (1% of an overnight culture) in MRS broth with 0.0, 0.4 and 0.6% phenol were tested. The media were then incubated at 37°C for 24 h and OD measured spectrophotometrically at 600 nm (Krausova *et al*., 2019).

Survival in artificial gastrointestinal juice: To determine the survival of *L. casei* NCDC 357 in gastrointestinal tract, the strain was exposed to artificial gastrointestinal juice *in vitro*. The strain

suspensions in phosphate buffer saline) (PSB: 0.2 mL) were added in a mixture of gastric juice pepsin (3 mg mL^{-1}, pH 2) (Zarate *et al*., 2006). Growth of strain in the same buffer adjusted to neutral pH was used as a control. The strain was then incubated at 37°C at 120 rpm and the viable count determined by plating MRS agar plates for 0, 1, 2, 3 and 4 h incubation. Similarly, the survival of strain in simulated intestinal conditions was tested in pancreatin (1 mg mL^{-1}, pH 8) containing 0.5% w/v sodium chloride. The percentage survival was calculated by the formula:

Survival (%) = Colony forming units (CFU) (Final) × 100/ CFU (Initial)

Auto-aggregation assay: Auto-aggregation of *L. casei* NCDC 357 was studied as per Kaushik *et al.* (2009). Briefly, the washed cell pellets of freshly grown culture were suspended in phosphate-buffered saline buffer (PBS; pH 7.0) until the absorbance of 0.6 at 600 nm was attained. The cell suspension was incubated at 37°C undisturbed. Then 1 mL suspension was drawn carefully, and absorbance measured at 600 nm using a spectrophotometer (Systronics, India) after 1, 2, 3, and 4 h. Percentage auto-aggregation was calculated using the formula:

$$\text{Auto-aggregation}\,(\%) = \left(\frac{\text{A-B}}{A}\right) \text{x } 100$$

where A is the absorbance at 0 h incubation and B is the absorbance after incubation for 1, 2, 3 and 4 h

Antibiotic susceptibility test

The sensitivity of *L. casei* NCDC 357 to antibiotics was assessed by disc diffusion method. For this, antibiotic rings (HiMedia) containing 6 antibiotics *viz*., ampicillin (AMP) 10 µg, amoxyclav (AMC) 30 µg, cefotaxime (CTX) 30 µg, cotrimoxazole (COT) 25 µg, gentamicin (GEN) 10 µg and tobramycin (TOB) 10 µg were tested to examine the sensitivity pattern of isolate. The isolate was thoroughly spread on nutrient agar plates and kept as such for 5 min to dry. Then, the antibiotic discs were placed on dried agar plates and incubated for 24 h at 37°C. The zones of inhibition were measured and compared to the values proposed by Charteris *et al.* (1998) to score strains as resistant, moderately sensitive and sensitive.

Evaluation of antidiabetic and antioxidant activities

The antidiabetic activity of *L. casei* NCDC 357 was measured using cell free supernatant (CFS) as per Won *et al*. (2021). For this purpose, the strain was cultured in MRS broth at 37°C for 18 h, centrifuged at 8000 rpm for 15 min at 4°C, and then the supernatant was filtered through a 0.45 µm syringe filter. Acarbose was used as a reference drug while determining the antidiabetic activity.

α-amylase inhibitory activity: The α-amylase inhibitory activity of LAB was evaluated as described by Vankudre *et al.* (2015). Briefly, 100, 200, 250 and 500 µL CFS were added to 400 µL of α-amylase solutions (0.5 mg mL^{-1}) and pre-incubated at 25°C for 10 min. The reaction mixture was then incubated with 400 µL starch solution (1% w/v in 0.02 M sodium phosphate buffer) at 25°C for 10 min. The reaction was terminated by adding 1000 µL of DNS colour reagent. The reaction mixture was boiled for 5 min, cooled, and diluted 4-fold with water. The absorbance was measured spectrophotometrically at 540 nm. The inhibition was calculated as follows:

$$\text{Inhibition}\,(\%) = \left(\frac{\text{A - B}}{A}\right) \text{x } 100$$

where *A* is the absorbance of control (with α-amylase and without samples) and *B* is the absorbance of sample

α-glucosidase inhibitory activity: α-glucosidase inhibitory activity of strains was evaluated as per the method of Chen *et al.* (2014). Briefly, 50, 100, 250 and 500 Ml CFS were added to the reaction mixture containing 150 µL of 0.01 M PBS (pH 7.0) and 75 µL of 0.02 M PNPG solution and pre-incubated at 37°C for 10 min. The reaction was initiated by adding 50 µL α-glucosidase, and the sample was incubated at 37°C for 10 min. The reaction was terminated by adding 1 mL of 0.1 M Na_2CO_3. The amount of p-nitrophenol released was determined by measuring the absorbance at 405 nm using a spectrophotometer. The inhibition was calculated as follows:

$$\text{Inhibition } (\%) = \left(\frac{1 - (C\text{-}D)}{(A - B)}\right) \text{x } 100$$

Where *A* is the absorbance with α-glucosidase but without the sample, *B* is the absorbance with neither α-glucosidase nor the sample, C is the absorbance with α-glucosidase, and the sample, and D is the absorbance without α-glucosidase but with the sample

Antioxidant activity

The DPPH (2,2–diphenyl–1–picrylhydrazyl) radical scavenging ability of *L. casei* NCDC 357 was determined as per the method of Kao *et al.* (2006) with some modifications. Briefly, 100, 200, 500 and 1000 µL CFS of *L.casei* NCDC 357 was added to 2.0 mL ethanolic DPPH radical solution (0.04 mM). The mixture was mixed vigorously and incubated at room temperature in dark for 30 min. The controls included deionized water and DPPH solution. The absorbance was measured at 517 nm spectrophotometrically (Systronics, India) in triplicate. The scavenging ability was calculated as:

$$\text{Scavenging effect } (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \text{x } 100$$

where A_1 is the absorbance of the test sample, and A_0 is the absorbance of the control at 517 nm

For measuring the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity, the method of Re *et al.* (1999) was followed. Briefly, the radical cation solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate (1:1 v/ v) and kept in dark for 24 h at 20-22°C. Then, 50, 100, 200 and 300 µL CFS of *L. casei* NCDC 357 and 450 µL ABTS solution were mixed and incubated for 10 min at room temperature in dark. The absorbance of mixture was measured at 734 nm. Sample assay was performed in triplicate, and the scavenging activity was calculated using the following formula:

$$\text{Scavenging rate } (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \text{x } 100$$

Where A_1 is the absorbance of the test sample, and A_0 is the absorbance of the control at 734 nm

Antagonistic activity against pathogens

Agar well diffusion assay method was used to detect the inhibitory properties of *L. casei* NCDC 357 against 7 pathogens procured from Microbial Type Culture Collection (MTCC), Chandigarh (India). Briefly, 24-48 h old culture of *L. casei* NCDC 357 was centrifuged for 10 min at 10,000 rpm. The supernatant was separated and tested against 7 test pathogens, *viz*., *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC439), *Klebsiella pneumoniae* (MTCC109), *Micrococcus luteus* (MTCC 950), *Pseudomonas aeruginosa* (MTCC 424), and *Staphylococcus aureus* (MTCC96). After 24 h incubation, the inhibition zones around the wells were measured.

Statistical analysis

All the experiments are performed in triplicate and the results presented as the means ± standard deviation (SD). The IC_{50} value, that is half maximal inhibitory concentration, was calculated from R^2 equation obtained from the linear thread line from the respective graph of concentration of CFS/ standard against % inhibition values.

RESULTS AND DISCUSSION

Evaluation of probiotic properties

Probiotics must survive in the human gastrointestinal conditions. Consumed LAB in stomach comes in contact with gastric juice, bile salt and enzymes and finally get attached to the intestine.

Bile, acid, and phenol tolerance

L. casei strain NCDC 357 could survive at different pH concentrations, bile, and phenol with moderate to a maximum survivability (Tables 1-3). A lactic acid bacterium (LAB) can be considered probiotic only if it has the characteristic ability to overcome the effect of bile salt and low environmental pH of gastrointestinal tract, especially in humans. Bile salt plays a crucial role in the defense mechanism of intestinal tract. Hence, the possession of bile tolerance by a LAB is considered beneficial. *L. casei* NCDC 357 strain showed resistance to phenol at the bile concentrations of 0.4 and 0.6% (w/v). The survival of LAB strains at 0.4 and 0.6% phenol has previously been reported (Mannan *et al.*, 2017). Phenol is a known toxic metabolite produced during digestion in the intestinal tract (Rowland *et al.*, 2018). Hence, probiotic bacteria should be able to tolerate phenol inside gastrointestinal tract of host (Vankudre *et al*., 2015).

Table 1: Bile tolerance rate of *L. casei* NCDC 357

Bile salt (%)				Survival rate
0.0	0.2	0.4	0.6	at 0.6%
9.0 ± 0.05	8.6 ± 0.20	7.8 ± 0.10	7.0 ± 0.11	78.3%

Table 2: Acid tolerance and survival rate of *L. casei* NCDC 357 under acidic condition

pH	Viable cells (log CFU mL^{-1})		Survival rate
	0 h	3 h	(%)
2.0	9.0 ± 0.15	5.4 ± 0.11	60.5
4.0	9.0 ± 0.15	7.9 ± 0.15	87.8
6.0	9.0 ± 0.15	8.5 ± 0.20	94.8

Table 3: Tolerance of *L. casei* NCDC 357 to phenol

Phenol	Viable cells (log CFU mL^{-1})		Survival rate
(%)	0 h	24 h	(%)
0.4	9.1 ± 0.01	8.8 ± 0.10	96.7
0.6	9.1 ± 0.20	8.0 ± 0.20	88.3

For consideration as a probiotic, LAB must survive in transit through stomach where pH is as low as 1.5 to 2.0 and reach the colon as physiologically viable active cells. In present study *L. casei* NCDC 357 was exposed to acidic pH 2, 4, and 6 to check its survival efficiency. The strain showed > 60% survival at pH 2, >87% at pH 4 and >94% survival at pH 6 after 3 h incubation. The findings are in line with Maryam and Abubakr (2018) who found 68-88% survival of LAB cultures at pH 2, isolated from breast milk.

Tolerance to artificial gastrointestinal juice

Probiotic microbes should tolerate the pH 3, which is the pH of gastric mucus layer. In present study, the viability of *L. casei* NCDC 357 was reduced to 49% after 4 h exposure to gastric condition (Table 4). The survival ability of *L. casei* NCDC 357 in simulated intestinal fluid revealed high viability of 90% after 4 h incubation at 37˚C. The findings are in line with Bindu and Lakshmidevi (2021) who found temporary damage of probiotic cells for various bacterial strains.

Table 4: Tolerance of *Lactobacilluscasei* NCDC 357toartificial gastrointestinal juice

Exposure time (h)	SGF (pH 2.0)		SIF (pH 8.0)	
	Viable cells (log CFU mL^{-1})	Survival rate (%)	Viable cells (log CFU mL^{-1})	Survival rate (%)
0	9.0 ± 0.20	100.0	9.0 ± 0.20	100.0
1	5.8 ± 0.10	64.2	8.7 ± 0.20	97.4
2	5.3 ± 0.05	59.0	8.6 ± 0.15	95.9
3	5.0 ± 0.10	55.4	8.4 ± 0.20	93.3
4	4.5 ± 0.20	49.4	8.2 ± 0.15	90.7

SGF: Simulated gastric fluid, SIF: Simulated intestinal fluid

Auto-aggregation assay

Auto-aggregation reveals the self-interaction of a culture in non-specific way which is considered a prerequisite for epithelial colonization. The current study revealed that *L. casei* NCDC 357 had 64.7% auto-aggregation after4 h incubation (Table 5). The findings are in line with de Souza *et al*. (2019) who found 78% auto-aggregation in *L. casei* strain SJRP135. The present study supports the

Table 5: Auto-aggregation assay of *L. casei* NCDC 357

Percent auto-aggregation			
1 h	2 h	3 h	4 h
29.5 ± 1.36	39.3 ± 1.64	54.2 ± 1.83	64.7 ± 1.92

findings of García-Cayuela *et al.* (2014) who reported more than 50% auto-aggregation rate in several probiotic strains.

Antibiotic susceptibility

The *L. casei* strain NCDC 357 was sensitive to cefotaxime and cotrimoxazole but resistant to ampicillin, amoxyclav, gentamicin and tobramycin. These findings are in agreement with Sharma *et al*. (2017) who reported that various *L. casei* strains isolated from milk had sensitivity to antibiotic cefotaxime. The present study showed that antibiotic resistance is widespread among different lactobacilli, thus indicating a food safety concern. The antibiotic sensitivity should be considered as a key tool for safety valuation of probiotics.

α-amylase and α-glucosidase inhibitory activities of L. casei NCDC 357

α-glucosidase is an enzyme in brush border membrane that catalyzes carbohydrate digestion. The inhibition of α-glucosidase activity has been demonstrated to prevent T2DM by decreasing glucose absorption and reducing the blood glucose levels (Marella *et al*., 2020). Acarbose, an antidiabetic, is most commonly used α-glucosidase inhibitor to treat diabetics and acts by delaying the glucose release from polysaccharides by binding to α-glucosidase. Various reports have suggested the beneficial role of α-glucosidase and α-amylase inhibitory activity of probiotics in glycemic regulation (Gazza *et al*., 2016). Probiotics reduce T2DM by controlling glucose metabolism and revamping the insulin sensitivity by many mechanisms, such as metabolite production (Morrison and Preston, 2016; Herrema and Niess, 2020). Various probiotics reportedly inhibit α-glucosidase activity by producing bioactive compounds (Zeng *et al*., 2016). In present study, α-glucosidase and α-amylase inhibitory activities were slightly less than acarbose. The α-glucosidase and α-amylase inhibition rates of *L. casei* NCDC 357 were 67.7% with IC_{50} value of 38.07 μL mL^{-1} and α-amylase 53.6% with IC_{50} value of 49.96 μL mL^{-1}, respectively; while the inhibitory activities of acarbose were 63.5% with IC_{50} value of 104.96 μg mL^{-1} and 74.3% with IC_{50} value of 109.09 μg mL^{-1} against α-glucosidase and α-amylase (Table 6). These findings are in line with Wang and Li (2022) who reported 28.8% α-glucosidase activity by a strain of *L. casei*. Koh *et al.* (2018) reported 37.9 and 39.0% α-glucosidase activity for culture of LGG and *Liquorilactobacillus mali* K8, respectively. Thus, the present findings demonstrate that *L. casei* NCDC 357 exhibit antidiabetic activity by inhibiting the carbohydrate metabolizing enzymes.

Table 6: Antidiabetic activity of *L. casei* NCDC 357

α-glucosidase					
Acarbose (μg mL^{-1})	Inhibition (%)	IC_{50}	*L. casei* NCDC 357 (μL)	Inhibition (%)	IC_{50}
100	36.0 ± 1.51		50	30.5 ± 0.66	
250	42.5 ± 0.24		100	41.4 ± 1.14	
500	53.1 ± 0.77		250	56.6 ± 0.25	
1000	63.5 ± 0.25	104.96	500	67.7 ± 0.43	38.07
α-amylase					
Acarbose (μg mL^{-1})	Inhibition (%)	IC_{50}	*L. casei* NCDC 357 (μL)	Inhibition (%)	IC_{50}
100	48.1 ± 1.08		100	24.9 ± 0.86	
250	54.1 ± 0.36		200	33.7 ± 1.32	
500	63.6 ± 0.73		250	42.0 ± 0.91	
1000	74.3 ± 0.72	109.09	500	53.6 ± 0.84	49.96

Antioxidant activity

Oxidative stress is induced by imbalance between ROS production and antioxidant defense mechanisms (Kumar *et al*., 2015). LAB produce bioactive compounds with beneficial antioxidant activity that may be rendered by specific molecular mechanisms responsible for defense against

oxidative stress based on the strain specificity. LAB produce various enzymes, such as oxidase, catalase or superoxide dismutase to eliminate H_2O_2 and O^-_2 **(**Wang *et al*., 2017). The antioxidant activity of *L. casei* NCDC 357 was tested by DPPH and ABTS radical scavenging activity. In present study the DPPH free radical scavenging activity was 53.4% with IC_{50} value of 77.60 µL mL^{-1} and the ABTS radical scavenging activity was 48.7% with IC_{50} value of 51.48 µL mL^{-1} for *L. casei* NCDC 357; while the antioxidant activity of ascorbic acid was 89.18% with IC_{50} 4.42 µg mL^{-1} and 41.05% with IC_{50} 10.42 µg mL^{-1} for DPPH and ABTS radical scavenging activities, respectively (Table 7). These findings are in line with Saadatzadeh *et al.* (2013) wherein strain *L. casei* ATCC 39392 showed 12-30% DPPH activity for various grades of supernatant. Similarly, 31-48% DPPH radical scavenging activities were reported for *L. acidophilus* and *L. paracasei* strains (Lin *et al*., 2019)**.** The findings demonstrate that *L. casei* NCDC 357exhibits significant antioxidant activity.

Table 7: Antioxidant activity of *L. casei* NCDC 357

Antioxidant activity					
Ascorbic acid (µg mL^{-1})	DPPH scavenging (%)	IC_{50}	*L. casei* NCDC 357 (µL)	DPPH scavenging (%)	IC_{50}
10	21.13		100	15.70 ± 0.55	
25	51.61		200	25.87 ± 0.67	
50	68.38		500	41.37 ± 0.68	
100	89.18	4.42	1000	53.40 ± 0.81	77.60
L. casei NCDC 357 (µL)	ABTS scavenging (%)	IC_{50}	*L. casei* NCDC 357 (µL)	ABTS scavenging (%)	IC_{50}
10	14.96 ± 0.52		50	32.81 ± 1.25	
25	20.73 ± 0.69		100	40.70 ± 0.77	
50	33.27 ± 1.05		200	45.71 ± 0.71	
100	41.05 ± 1.27	10.48	300	48.70 ± 0.51	51.48

Antagonistic activity against pathogens

The antimicrobial activity of LAB is essential because these bacteria must inhibit and kill the growth of pathogenic microorganisms in food products and intestinal environments. The anti-microbial effect of LAB can be attributed to metabolites such as organic acids, hydrogen peroxide, diacetyl, ethanol, phenols, and protein that is produced during growth. Bacteriocin, organic acids (especially lactic and acetic acids) and hydrogen peroxide are the main compounds produced by probiotics. The metabolites and competitive exclusion mechanism of probiotics for adhesive receptors and nutrients together could inhibit the pathogenic growth inside the host (Barzegar *et al*., 2021). The *L. casei* NCDC 357, tested for antimicrobial activity against 7 selected pathogens, showed strong broad spectrum inhibitory activities against *E. coli, B. subtilis, S. aureus, E. faecalis, K. pneumoniae, P. aeruginosa,* and *M. luteus* (Table 8). *L. casei* NCDC 357 showed remarkable antimicrobial against pathogenic microbes. The strain showed highest zone of inhibition (21.9 mm) against *P. aeruginosa*, while antibiotic ampicillin showed a maximum inhibition zone of 24.7 mm against *E. coli*. The present findings revealed that *L. casei* NCDC 357 strain has broad-spectrum antimicrobial activities and the results are comparable with standard antibiotic drugs.

Table 9: Antagonistic activity of *Lactobacillus casei* NCDC 357 against pathogens in comparison to ampicillin

Pathogen(s)	*L. casei* NCDC 357 (70 µL)	Ampicillin (20 µg L^{-1})
Bacillus subtilis	15.6 ± 0.90	22.6 ± 0.80
Staphylococcus aureus	17.5 ± 0.96	20.8 ± 1.63
Escherichia coli	20.3 ± 0.62	24.7 ± 0.81
Klebsiella pneumoniae	18.0 ± 1.30	22.6 ± 1.00
Enterococcus faecalis	13.7 ± 0.58	23.5 ± 1.32
Micrococcus luteus	11.1 ± 0.46	24.4 ± 1.00
Pseudomonas aeruginosa	21.9 ± 1.23	19.9 ± 1.22

The present study revealed that the probiotic *L. casei* strain

NCDC 357 has remarkable antidiabetic and antioxidant abilities. The strain has potential to combat various food pathogens. However, further studies are desired to confirm potential health benefits and applications.

Conflicts of interest: The authors declare no conflict of interest.

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