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Original article

Antagonistic, Anti-oxidant, Anti-inflammatory and Anti-diabetic Probiotic Potential of *Lactobacillus agilis* Isolated From the Rhizosphere of the Medicinal Plants

Allah Nawaz Khan [a,b], Humaira Yasmin [a,*], Shakira Ghazanfar [c], Muhammad Nadeem Hassan [a], Rumana Keyani [a], Imran Khan [c], Madeha Gohar [a], Asim Shahzad [d,e], Maha J Hashim [f], Ajaz Ahmad [g]

[a] *Department of Biosciences, COMSATS University Islamabad (CUI), Islamabad, Park road, Chak shazad, 44000 Islamabad, Pakistan*
[b] *Department of Allied Health Sciences, Iqra University Islamabad Campus, Chak Shahzad, Pakistan*
[c] *Functional Genomics and Bioinformatics Centre (FGBC), National Institute for Genomics Advanced Biotechnology (NIGAB) National Agricultural Research Centre, (NARC) PARC, Park Road, Islamabad 45500, Pakistan*
[d] *Department of Botany Mohi.ud.Din Islamic University, Nerian Sharif, AJK, Pakistan*
[e] *The College of Geography and Environment Science" Henan University, Kaifeng , China*
[f] *Department of Biosciences, University of Nottingham, Nottinghamshire, UK*
[g] *Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

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ABSTRACT

Potential probiotic bacteria can be used as a biotherapeutic agent and a sustainable alternative to antibiotics, as an anti-oxidative, anti-inflammatory, and anti-diabetic agent without causing any serious side effects. Mostly human-friendly Lactic acid bacteria (LAB) have been isolated from the animal-human origin to be used as biotherapeutic agents or to produce useful metabolites (nutraceutical). However, less information is known about the role of medicinal plants associated LAB as biotherapeutic agents. The isolation of 115 human-friendly *Lactobacillus* strains was done from the rhizosphere of the medicinal plants *Ocimum tenuiflorum, Azadirachta indica, Ficus carica*. The obtained bacteria were then tested for their safe status before being using it for a beneficial purpose. Out of 115 strains, 29 (25%) were negative for blood hemolytic activities. Among these 29 isolates, three isolates did not show a breakdown of gelatin and were recognized as safe. Antibiotic resistance assay showed resistance of two of them against antibiotics discs of Streptomycin (10 μg), Ciprofloxacin (20 μg), Vancomycin (30 μg), Metronidazole (10 μg), Ampicillin (5 μg), Chloramphenicol (30 μg), Kanamycin (30 μg), Erythromycin (15 μg), Penicillin (10 μg) and Tetracycline (30 μg). The bacterial isolate (T-2) was found safe that was identified as *Lactobacillus agilis* by sequence analysis of 16 s rRNA gene and processed *in vitro* as an anti-bacterial, anti-oxidant, anti-diabetic, and anti-inflammatory agent. Free radical scavenging activities and inhibition of α-amylase activities for *Lactobacillus agilis* were found relative to standard drug values as 68% and 73% and 51.3% and 65.3%, respectively. The *in-vitro* anti-inflammatory assay showed 61.6% (*Lactobacillus agilis*) while showed 69% (aspirin) activity for denaturation albumin protein. The results suggested that *Lactobacillus agilis* can be used as a potential probiotic strain as well as can be used to produce nutraceuticals.

1. Introduction

Probiotics are used as food additives that have beneficial effects on the healthy body by setting microbial balance in the gastrointestinal tract. According to scientific reports, probiotics have anti-allergic and anticancer effects, cholesterol-lowering effects, improvement of host immune response, treating irritable bowel syndrome and improving intestinal inflammation (Shokryazdan et al., 2014). These probiotics strains have been collected directly from the gastrointestinal tract (GIT) or other sources such as feces

* Corresponding author.
E-mail addresses: humaira.yasmin@comsats.edu.pk (H. Yasmin), nadeem_hassan@comsats.edu.pk (M.N. Hassan).
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and milk. The most common organism used in the essential preparation of probiotics is the lactic acid bacteria (LAB), which is also part of the natural flora of organism GIT system and is considered safe according to Food and Drug Administration (FDA) (Fijan, 2014). They are a group of non-sporulating, Gram-positive, anaerobic, or facultative aerobic, cocci or rods, which produce lactic acid (Zheng et al., 2020).

The LAB helps to induce an immune response and reduce/inhibit the pathogens through several mechanisms. Many studies reported α-glucosidase inhibition activity (Khan et al., 2017), production of bacteriocins (protein compounds) which have antagonistic potential against pathogenic bacteria, and a different degradation system in the digestive system (Hawaz, 2014). Hernández-González et al. (2021) also studied the antimicrobial, probiotics, and immunomodulatory potential of LAB in veterinary medicine. Moreover, several studies have documented the antibacterial effects of probiotics as antagonistic bacteria against Gram-negative and Gram-positive pathogenic bacteria such as *E. coli*, *P. aeruginosa and S. aureus* and proved that probiotics can be used as antimicrobial agents instead of antibiotics to minimize antibiotics resistance (Sharafi et al., 2013).

Diabetes mellitus is caused by insulin deficiency, hyperglycemia and sugar, fat, and protein metabolism dysfunctions (Vieira et al., 2019). The data from 1980 to 2014 shows a worldwide increase in diabetes from 108 million to 422 million respectively. Global prevalence rose from 4.7% in 1980 to 8.5% in 2014. In 2012, 2.2 million deaths were reported before age of 70 due to hyperglycemia in blood similarly in 2016, approximately, 1.6 million death were caused by diabetes (World Health Organization, 2017). Additional health problems experienced by diabetic patients like blindness, kidney failure, heart attacks, amputation of the lower limb (Papatheodorou et al., 2017).

Type-2 diabetes happens due to an increase in blood glucose level and reactive oxygen species (ROS), which leads to cell malfunctioning and insulin resistance (Asmat et al., 2016; Hannoodee & Nasuruddin, 2020). According to the World Health Organisation (WHO) report, about 70–80% of the world's population rely on non-conventional medicine mainly from herbal sources. Its demand is increasing gradually in developing countries (Wirtz et al., 2017). Therefore, the use of safe, natural, and economic alternatives like the use of probiotics can be used to cope with these issues. Chen et al. (2020) evaluated the safe status of *Lactococcus lactis* and *Leuconostoc lactis* strains and further examined the strains via *in vitro* tests for antimicrobial activity, cell surface characteristics, heat treatment, antibiotic susceptibility, and acid/bile tolerance.

A survey of the literature showed no systemic approach has been made to evaluate the antibacterial, antioxidant, anti-inflammatory and anti-diabetic potential of *Lactobacillus agilis in vitro*. The present study involves the determination of the antibacterial activity of *L. agilis* by growth inhibition of the pathogenic bacteria, antioxidant by scavenging potential of free radical, anti-inflammatory activity by inhibition of albumin denaturation and anti-diabetic potential by inhibition α. amylase activity. This study was aimed at the isolation of safe *Lactobacillus* species from the rhizosphere of the *Ocimum tenuiflorum* (Tulsi), *Azadirachta indica* (Neem), *Ficus carica* (Anjeer), which were tested *in vitro* for their beneficial effects.

2. Materials and methods

2.1. Sample collection

Rhizospheric soil (10-–20 cm depth) samples of *Ocimum tenuiflorum* (Tulsi), were collected from Bannu (32.9910° N, 70.6455° E), Bhakkar (31.6082° N, 71.0854° E), Islamabad (33.6844° N, 73.0479° E), Mian wali (32.5839° N, 71.5370° E) and Multan (30.1575° N,71.5249° E) (03 from each site) in March. The rhizosphere soil of *Ficus carica* was obtained from Bannu, Mian wali and Muree (33.907774° N, 73.3915° E) while, soil samples of *Azadirachta indica* (Nim/ margosa/ nimtree/ indian lilac) were collected from Islamabad, Mian wali, Bakhar and Sahiwal (30.677717° N, 73.106812° E). These samples were stored in zipper bags and transported to lab in ice for further processing.

2.2. Isolation of Lactobacillus from the rhizosphere of medicinal plants

Each (1 g) of soil sample collected from the rhizospheric regions was serially diluted up to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. Dilutions were spread (50 μL) on MRS agar media (selective for *Lactobacillus* species) (De Man et al., 1960). Plates were incubated for 24 h at 32 °C. After incubation, individual colonies were again subcultured on MRS agar and incubated at 32 °C for 24 h to get pure colonies of each isolate. All the obtained isolates were observed for colony morphology and Gram staining was performed.

2.3. Characterization of Lactobacillus isolates for their safe status

Safe status of the isolated bacterial strains was confirmed by the following assays before using it for useful purpose.

2.3.1. Blood hemolytic test

Blood hemolytic assays according to the protocol of Harrigan were performed (Harrigan, 1998). Colonies were inoculated on the blood agar media and plates were incubated for 24 h at 32 °C. After incubation, the bacterial colonies were checked for blood hemolysis forming a clear zone on media.

2.3.2. Gelatinase and DNase tests

Gelatinase and DNase tests were performed by culturing the bacterial colonies on gelatin agar media and DNase agar media for 24 h at 32 °C (Gupta & Malik, 2007). The isolates positive for gelatinase and DNase enzyme showed clear zones on media.

2.3.3. Antibiotic resistance assay

Antibiotic resistance assays were performed by disc diffusion method according to the protocol of Thirabunyanon et al., (2009). The three isolates were spread on Muller Hinton agar plates separately. Antibiotics discs of Streptomycin (10 μg), Ciprofloxacin (20 μg), Vancomycin (30 μg), Metronidazole (10 μg), Ampicillin (5 μg), Chloramphenicol (30 μg), Kanamycin (30 μg), Erythromycin (15 μg), Penicillin (10 μg) and Tetracycline (30 μg) were used against each isolates. These assays were done in triplicates after incubation for 24 h at 37 °C. The selection was done based on the zones diameter. The larger zone (mm) area showed sensitive strain (no resistance) while smaller zone area meant more resistance. The isolates showing high percentage of resistance to antibiotics were also excluded.

2.4. Molecular identification

Potential isolates of the bacteria were selected based on the above-performed assays. For molecular identification, the genome of the isolate was extracted through the CTAB method. According to the protocol of Ahmad et al. (2008) universal primer P1 (5′-PA GAGTTTGATCCTGGTCAGAACGAACGCT-3′) and P6 (5′-TACGGC TACCTTGTTACGACTTCACCCC – 3′), was used to amplify the 16S rRNA gene. The amplified product of the isolate was sequenced by MACROGEN (Seoul, Korea). The gene sequences were then assembled using BioEdit software as described by Hall (Hall,

1999). The identified sequences were submitted for accession number to NCBI (National center for biotechnology information).

2.5. Phylogenetics analysis

Evolutionary analyses were conducted in MEGA X according to Kumar et al (2018). To construct a Phylogenetic tree, the sequence was Blast for the most similar sequences. The most similar sequences were retrieved from NCBI in FASTA format and aligned using Mega X software. Mega X was used for tree construction using MSA data. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nai, 1993).

2.6. In vitro assays of L. agilis NMCC-15

2.6.1. Antibacterial properties

Antagonistic activity of *L. agilis* NMCC-15 was determined against pathogenic strains of *Escherichia coli* (ATCC8739), *Pseudomonas aeruginosa* (ATCC9027), *Staphylococcus aureus* (ATCC6538), *Listeria monocytogenes* (ATCC13932) and *Bacillus cereus* (ATTCC11778). The screened isolates were cultured on MRS broth at 35 °C for 5 days on a rotary shaker. Antibacterial assay of the crude supernatant from culture was done using the well diffusion method (Kimura et al., 1998). The pathogenic cultures were spread on Muller Hinton agar (MHA) media. MHA plates were then punched using a sterilized cork borer to make wells at equal intervals. Thereafter, 150 μL of each rhizobacterial supernatant from fermented culture was poured into wells. Plates were incubated at 35 °C ± 2 for 24 h. This assay was done in triplicates. Zones of inhibition in millimeters were measured as mean values. According to the diameter of the inhibition zone, the antipathogen activity was classified into strong (diameter $\geq$ 20 mm), moderate (diameter > 10 mm), and weak (diameter $\leq$ 10 mm).

2.6.2. Antioxidant activity

Antioxidant activity of *L. agilis* NMCC-15 was tested by using DPPH (2,2-diphenylpicrylhydrazyl) as a free radical using the method of (Farzana et al., 2011). For free radical solution, DPPH of 0.003 g was dissolved in 100 mL of methanol. The volume of 200 μL from the supernatant (*L. agilis* broth culture) was added to 800 μL of DPPH solution. The same solution was made for ascorbic acid as standard. Both the solution was then kept for 30 min at 37 °C. Scavenging or the inhibition of free radicals by the supernatant and ascorbic acids were measured at 517 nm by a UV–visible spectrophotometer. This assay was repeated three times and the mean value was calculated by using the given formula:

$$\text{Inhibition (\%)} = [(\text{Absorbance of control} - \text{Absorbance of tests})/\text{Absorbance of control}] \times 100$$

2.6.3. Antidiabetic activity

Antidiabetic activity (Inhibition of α-amylase activity) of *L. agilis* NMCC-15 (MK614016) was done through α-amylase inhibition assay by following the method of Sathiavelu et al. (2013). Volume 500 μL of supernatant (*Lactobacillus agilis* broth culture) was added to 500 μL volume of phosphate buffer solution containing α-amylase. The same solution was made for sitagliptin as standard. After incubation for 10 min at 25 °C, both the mixtures were treated with dinitrosalicylic acid (DNSA). Treated mixtures were kept at boiling point in the water bath for 5 min and then cooled at room temperature. Thereafter, 10 mL of dH_2O was added to both mixtures, and absorbance was measured for both mixtures at 540 nm using UV–visible spectrophotometer. This assay was repeated three times and the mean value was calculated. Percentage inhibition% was calculated by the method of Oboh et al., (2012).

$$\text{\% Inhibition} = [(\text{Absorbance of Control} - \text{Absorbance of tests})/\text{Absorbance of Control}] \times 100$$

2.6.4. Anti-inflammatory activity

Anti-inflammatory activity of the *L. agilis* NMCC-15 was performed *in vitro* with the modified protocol of Sakat et al., (2010) by inhibition of albumin denaturation assay. A reaction mixture of the culture supernatant (500 μL/mL) and an aqueous solution of bovine albumin fraction (1%) was prepared. In the same ratio, a mixture of aspirin was prepared as standard. The mixtures were kept at room temperature for 20 min and then were heated up to 51 °C for 20 min to induce inflammation. The mixtures were then allowed to cool down and then turbidity of albumin protein was measured in both the mixtures at 660 nm via UV–visible spectrophotometer. This assay was repeated three times and the mean value was calculated.

Percentage Inhibition% was calculated from the mean value as:

$$\text{\% inhibition} = [(\text{Absorbance of control} - \text{Absorbance of tests})/\text{Absorbance of control}] \times 100$$

2.7. Statistical analysis

Statistical analysis of the data set was performed using software Statistix version 8.1. The variation in the data set in triplicates was analyzed using the technique ANOVA (analysis of variance). Standard error was calculated between three replicates of each treatment. Comparison between the treatments was done using the least significant difference (LSD) at p = 0.05.

3. Results

3.1. Isolation

Initially, 115 isolates were obtained from the sub culturing of samples on MRS agar which were different biochemically and morphologically. Fig. S1 indicates the distribution of bacterial strains from the rhizosphere of *Ocimum tenuiflorum, Azadirachta indica, Ficus carica*. All were found to be Gram's-positive, catalase-negative and oxidase-positive. Morphologically, all the strains varied in their color (creamy/white), shapes (circular/ rods), surface with the surface (smooth/rough).

3.2. Safe status assays

To exclude potentially harmful bacterial strains, testing of safe status was done before selection of bacterial strains for human as probiotics. All the 115 isolates that were obtained after subculturing were negative for the DNase enzyme. These isolates were then tested for blood hemolytic properties, out of 115 strains 29 (25%) were negative for blood hemolytic activities and the 86 isolates showed blood hemolysis effects, therefore, these positive strains were excluded from the study. The 29 strains were then tested for the presence of gelatinase, out of which 26 isolates break down gelatin and such isolates were also excluded. In the end, three (03) isolates were recognized as safe and all these were isolated from rhizospheric samples of *Ocimum tenuiflorum*, having no such properties to degrade DNA, blood and gelatin (Fig. S2).

Table 1
Antibiotic resistance profiles of the bacterial strains isolated from the rhizosphere of medicinal plant.

Strain ID	Stre[a] (10 µg)	Cipr[b] (20 µg)	Van[c] (30 µg)	Metr[d] (10 µg)	Ampi[e] (5 µg)	Chlor[f] (30 µg)	Kana[g] (30 µg)	Eryt[h] (15 µg)	Peni[i] (10 µg)	Tet[j] (30 µg)
A-1	8 ± 0.94B	3.33 ± 0.94 FGH	6 ± 0.94 CD	5.83 ± 0.94 CDE	5 ± 0.94 CDEFG	5.17 ± 0.94 CDEF	4.17 ± 0.94 DEFGH	1.17 ± 0.94 I	3 ± 0.94 HI	3 ± 0.94 HI
T-2 (NMCC-15)	11.833 ± 0.94A	12.667 ± 0.94 A	13.5 ± 0.94 A	11.833 ± 0.94 A	13.667 ± 0.94 A	12.333 ± 0.94 A	11.833 ± 0.94 A	12.17 ± 0.94 A	13.17 ± 0.94 A	12.67 ± 0.94 A
N-3	6.00 ± 0.94CD	4.00 ± 0.94 EFGH	4.17 ± 0.94 DEFGH	4.83 ± 0.94 CDEFGH	4.83 ± 0.94 CDEFGH	6.17 ± 0.94 BC	5.83 ± 0.94 CDE	4.83 ± 0.94 CDEFGH	4.17 ± 0.94 DEFGH	3.17 ± 0.94 GH

[a] Streptomycin.
[b] Ciprofloxacin.
[c] Vancomycin.
[d] Metronidazole.
[e] Ampicillin.
[f] Chloramphenicol.
[g] Kanamycin.
[h] Erythromycin.
[i] Penicillin.
[j] Tetracycline, Alpha 0.05. Standard Error for Comparison 0.94. Critical T Value 2.0. Critical Value for comparison 1.87. There are 9 groups (A, B, etc.) in which the means, are not significantly different from one another.

3.3. Antibiotic susceptibility

The antibiotic susceptibility of the selected three isolates against the high consumption of clinically important antibiotics was assessed based on the formation of inhibition zones (Table 1). The results were expressed as to select the strain which shows less or no resistance to antibiotics. Table 1 shows that strain T-2 (NMCC-15) is more sensitive to antibiotics as compared to strain A-1 and N-3 and hence strain A-1 and N-3 were excluded.

3.4. Molecular analysis

The amplification and sequence analysis of the 16S rRNA gene of screened bacterial isolate NMCC-15 showed a partial 641 bp sequence length of the 16S rRNA gene. The sequence was BLAST in NCBI which showed 99.69% to *L. agilis* strain JCM 1187 16S ribosomal RNA (Fig. 1). The strain was submitted to GenBank as *L. agilis* strain NMCC-15 16S ribosomal RNA gene, partial sequence. The accession no. obtained for *L. agilis* strain NMCC-15 was MK614016.

3.5. Evolutionary analysis by maximum likelihood method

The tree with the highest log likelihood (−143025.57) is shown. Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with a superior log-likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 101 nucleotide sequences. There were a total of 1573 positions in the final dataset. The tree shows that *L. agilis* NMCC-15 is more closely related to *L. hordei* strain UCC128, *L. oligofermentans* strain AMKR18, *Pediococcus parvulus* strain S-182 and *L. herbarum* strain TCF032-E4 (Fig. 2).

3.6. In-vitro antibacterial assay

The antibacterial assays showed the efficacy of the *L. agilis* NMCC-15 to kill or inhibit the growth of the bacterial pathogens *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes* and *Bacillus cereus* (Fig. 3). The antibacterial results showed the significant marked antagonistic activity of *L. agilis* NMCC-15 to kill or inhibit the growth of all the tested bacterial pathogens. The *L. agilis* NMCC-15 showed maximum inhibitory activity against *Escherichia coli* and *Staphylococcus aureus.*

3.6.1. Antioxidant, anti-diabetic, and anti-inflammatory assays

Table 2 represents the results of the antioxidant, antidiabetic, and anti-inflammatory activities of *L. agilis* NMCC-15. Supernatant from the broth culture of *L. agilis* NMCC-15 showed varying effects on inhibition of free radical, α-amylase and albumin protein denaturation.

3.6.2. Antioxidant activity of L. agilis

Results of antioxidant assay suggested the potential of *Lactobacillus agilis* NMCC-15 as an antioxidant. Percentage inhibition of the free radical from the mean values showed the comparable effects of *L. agilis* supernatant to the standard drug with values 68% and 73%, respectively.

3.6.3. Anti-diabetic activities of L. agilis

Results obtained for percentage inhibition of the α-amylase activities by both the treatments (*L. agilis* supernatant and standard Sitagliptin drug) showed significant inhibition of α-amylase activities with 51.3 and 65.3, respectively. These results showed

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Lactobacillus agilis strain JCM 1187 16S ribosomal RNA, partial sequence	Ligilactobacillus agilis	1175	1175	100%	0.0	99.69%	1485	NR_113259.1
Lactobacillus agilis strain DSM 20509 16S ribosomal RNA, partial sequence	Ligilactobacillus agilis	1129	1129	100%	0.0	97.82%	1511	NR_044700.2
Lactobacillus satsumensis strain NRIC 0604 16S ribosomal RNA, partial sequence	Lactobacillus satsumensis	928	928	95%	0.0	93.97%	1556	NR_028658.1
Lactobacillus ruminis strain NBRC 102161 16S ribosomal RNA, partial sequence	Ligilactobacillus ruminis	911	911	97%	0.0	93.10%	1488	NR_041611.1
Lactobacillus oeni strain 59b 16S ribosomal RNA, partial sequence	Lactobacillus oeni	909	909	94%	0.0	93.75%	1556	NR_043095.1
Lactobacillus sucicola strain NRIC 0736 16S ribosomal RNA, partial sequence	Lactobacillus sucicola	907	907	94%	0.0	93.61%	1503	NR_112785.1
Lactobacillus mali KCTC 3596 = DSM 20444 strain NBRC 102159 16S ribosomal RNA, partial sequence	Lactobacillus mali KCTC 3...	904	904	93%	0.0	93.84%	1485	NR_112691.1
Lactobacillus aquaticus strain IMCC1736 16S ribosomal RNA, partial sequence	Lactobacillus aquaticus	904	904	93%	0.0	93.84%	1528	NR_115847.1
Lactobacillus uvarum DSM 19971 strain 8 16S ribosomal RNA, partial sequence	Lactobacillus uvarum DS...	900	900	93%	0.0	93.82%	1520	NR_115308.1
Lactobacillus salivarius strain JCM 1231 16S ribosomal RNA, partial sequence	Ligilactobacillus salivarius	898	898	94%	0.0	93.55%	1486	NR_112759.1

Fig. 1. BLAST results shows the similarity to *Lactobacillus agilis*.

that *L. agilis* inhibited the activities of the α-amylase (anti-diabetic potential) by 51.3% than control.

3.6.4. Anti-inflammatory activities of L. agilis

Results for the denaturation albumin protein (anti-inflammatory) showed the pronounced anti-inflammatory effects for both *L. agilis* supernatant and aspirin (standard drug). The supernatant of *L. agilis* showed 61.6% while aspirin showed 69% activity for denaturation albumin protein.

4. Discussion

Probiotics have been used as nutraceuticals but their safe status for human health must be assured before their use as ecofriendly gut bacteria. Plenty of probiotic strains are currently available in markets, using the accessible alternative resource to isolate and develop new enhanced probiotic strains with valuable medical significance is a successful way to develop new better probiotic strains (Halder et al., 2017). Information is still scanty about the potential of medicinal plants associated with *lactobacillus* strains as nutraceuticals. Therefore, the current study focuses on the isolation, molecular identification and characterization of medicinal plants associated with rhizobacteria for their safe status for human consumption. Moreover, the safe strains that showed less antibiotic resistance were identified as *L. agilis* NMCC-15 based upon 16S rRNA gene sequencing. To our knowledge, this is the first report for *L. agilis* NMCC-15 associated with the rhizosphere of medicinal plants for their significant potential as anti-inflammatory, anti-diabetic, antioxidant, and antagonist against lethal human pathogens.

The probiotic bacteria must be incapable of causing hemolysis and gelatin liquefaction in the human host. All the lactobacillus strains isolated from the rhizosphere of medicinal plants were measured by blood haemolytic test, gelatinase, DNase tests and antibiotic resistance assay to exclude the strains that are not safe. Somashekaraiah et al., (2019) excluded those lactic acid bacteria which possess blood haemolytic, gelatinase, DNase test properties.

In this study antibiotic sensitivity of the *L. agilis* NMCC-15 against the tested antibiotics Streptomycin (10 μg), Ciprofloxacin (20 μg), Vancomycin (30 μg), Metronidazole (10 μg), Ampicillin (5 μg), Chloramphenicol (30 μg), Kanamycin (30 μg), Erythromycin (15 μg), Penicillin (10 μg) and Tetracycline (30 μg) was noted which showed that the strain is not resistant to antibiotics.

According to the WHO/FAO (food and agriculture organisation), the true probiotics should present their antimicrobial actions particularly to the pathogens in the GI system. Probiotics have been used as growth promoters to replace the widely used antibiotics and synthetic chemical feed supplements (AFRC, 1989). *Lactobacilli* are highly competitive due to their production of several antimicrobial compounds such as organic acids, ethanol, diacetyl, carbon dioxide, hydrogen peroxide and bacteriocins (Reis et al., 2012).

Results exhibited the positive antibacterial activity of the *L. agilis* NMCC-15 against *E. coli, P. aeruginosa, S. aureus, Listeria monocytogenes* and *B. cereus*. The inhibitory characteristics of probiotics against pathogens can be considered as their most important health-promoting properties (Gerez et al., 2013). These results were in accordance with Prieto et al., (2012) who reported antibacterial activities of bacterial strains *B. cereus*, *B. licheniformis*, *B. subtilis* and *B. pumilus* isolated from marine plants against *P. aeruginosa*, *S. aureus*, *S. enterica*, *B. sp*., *Proteus vulgaris*, *E. coli*, and *S. typhimurium*. Similar, results were reported by Weschenfelder et al., (2018) who prepared a kefir and assessed its antagonistic activity against *S. aureus* and *E. coli*.

Antioxidant enzymes are regarded as important enzymatic defense systems against oxidative stress in LAB. *L. agilis* NMCC-15 also showed antioxidant properties against free radicals. In accordance to our finds, *L. plantarum* AR113, AR269, AR300, AR501, and *P. pentosaceus* AR243 exhibited strong resistance to hydrogen peroxide (Antolovich et al., 2002). In the current study we found strong anti-oxidant activities of *L. agilis* NMCC-15. Similar results were reported by Li et al., (2012) who reported the antioxidant potential of *L. plantarum* strains isolated from traditional Chinese fermented foods. The hydroxyl radical and DPPH scavenging activities of *L. plantarum* C88 at a dose of 10^{10} CFU/ml were maximum against hydrogen peroxide.

LAB is known to release polysaccharides which can prevent the activity of the enzyme α- glucosidase (Reuben et al., 2020). In this study, *L. agilis* NMCC-15 was *in vitro* screened for the effective anti-diabetic property. This showed that NMCC-15 strain can inhibit the activity of α-glucosidase. Muganga et al., (2015) have reported *L. Plantarum*, *L. acidophilus* with antidiabetic potential. Similarly, *L. sakei* was reported by Bajpai et al., (2016) and *L. brevis* by Son et al., (2017) for effective anti-diabetic properties.

These results further indicate the potential of *L. agilis* NMCC-15 to be used as anti-inflammatory agents. As reported that *L. casei* and *L. acidophilus* had very reliable anti-inflammatory activity and thus showed a substantial decrease in the paw thickness of rats (Amdekar et al., 2012). A study conducted by Ganji-Arjenaki and Rafieian-Kopaei (2018) also showed that many stains of *lactobacillus* exhibit the potential to treat inflammatory bowel disease. The results obtained from the study of *L. agilis* NMCC-15, suggest that the tested strain can produce secondary metabolites with good abilities as antimicrobial, antioxidant, anti-diabetic and

Fig. 2. Unrooted phylogenetic tree based on 16S rRNA comparisons showing the relationships of *Lactobacillus agilis* NMCC-15 to other *Lactobacillus* strains.

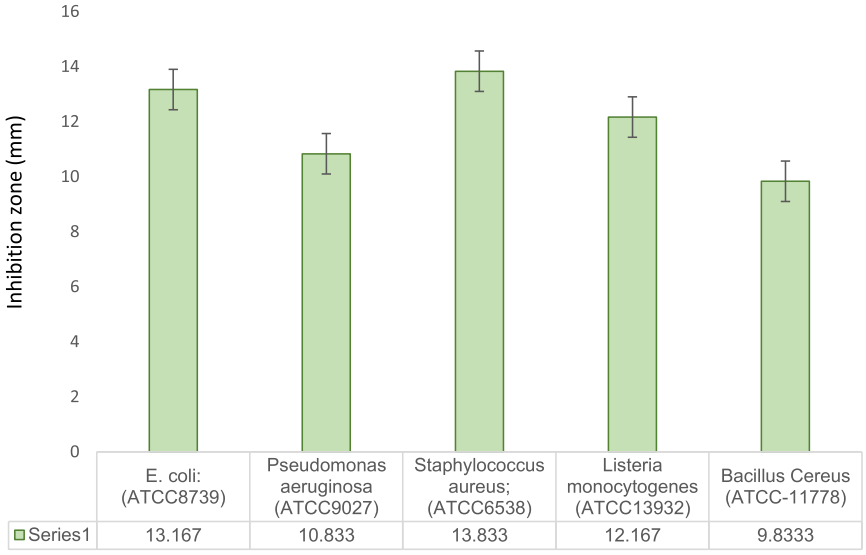

Fig. 3. Antagonistic activity of *Lactobacillus agilis* NMCC-15 against pathogenic bacteria and their zones diameter (mm). ATCC: American-type culture collection, Virgina, USA. Halo size > 8 mm (strong), 4–8 mm (moderate) and 1–4 mm (weak). Alpha 0.05. Standard Error for Comparison 0.8882. Critical T Value 2.042. Critical Value for Comparison 1.8139. There are 6 groups (A, B, etc.) in which the means are not significantly different from one another.

Table 2
In vitro antioxidant, antidiabetic, and anti-inflammatory activities of *L. agilis* in comparison to respective standard drug ascorbic acid, Sitagliptin and Aspirin, respectively.

In-vitro assay	Treatments	% inhibition
Antioxidant at 517 nm	Supernatant	68.000 ± 0.86B
	Ascorbic acid (drug)	73.333 ± 0.86 A
	Control	0 ± 0.86C
Antidiabetic at 540 nm	Supernatant	51.333 ± 1.0184B
	Sitagliptin (drug)	65.333 ± 1.0184 A
	Control	0 ± 1.0184C
Anti-inflammatory at 660 nm	Supernatant	61.667 ± 0.86B
	Aspirin (drug)	69 ± 0.86 A
	Control	0 ± 0.86C

Alpha 0.05. Standard Error for Comparison 0.8607. Critical T Value 2.447. Critical Value for comparison 2.1060. All 3 means are significantly different from one another.

anti-inflammatory. However, *in vivo* studies will be needed to confirm the potential of *L. agilis* NMCC-15 for their ability to be a potential biotherapeutic agent

5. Conclusions

L. agilis NMCC-15, potentially novel and safe rhizobacteria, showed significant antagonism against lethal human pathogens and positive activity as an anti-diabetic, anti-inflammatory, and antioxidant agent. *In vivo* studies will be needed to validate *L. agilis* NMCC-15′s potential as a biotherapeutic agent.

Ethics approval

Not Applicable.

Consent to participate

All authors consent to participate in this manuscript.

Consent for publication

All authors consent to publish this manuscript in the Saudi Journal of Biological Science.

Availability of data and material

Data will be available on request to the corresponding or first author.

Code availability

Not Applicable.

CRediT authorship contribution statement

Allah Nawaz Khan: Writing original draft, Methodology, Data curation, Formal analysis, Conceptualization. **Humaira Yasmin:** Review and editing, Conceptualization, Supervision, Project administration. **Shakira Ghazanfar:** Data curation, Formal analysis and Review and editing. **Muhammad Nadeem Hassan:** Formal analysis, Validation, Review and editing, Resources. **Rumana Keyani:** Data curation, Review and editing, Software, Data analysis. **Imran Khan:** Methodology, Formal analysis, Review and editing. **Madeha Gohar:** Review and editing, Softwares, Data analysis. **Asim Shahzadd:** Formal analysis, Review and editing, Resources. **Maha J Hashim:** Methodology, Visualization, Resources, Review and editing. **Ajaz Ahmad:** Methodology, Resources, Review and editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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