Role of *Lactiplantibacillus plantarum* strain RD1 (Lpb RD1) in mitochondria-mediated apoptosis: an *in vitro* analysis

Durga Rathikota1 · Surekha Kattaru3 · Venkata Gurunadha Krishna Sarma Potukuchi2 · Suneetha Yeguvapalli2

Received: 1 June 2022 / Revised: 18 July 2022 / Accepted: 7 August 2022 © The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

**Abstract**

The purpose of this study was to determine the cytotoxicity of *Lactiplantibacillus plantarum* strain RD1 (Lpb RD1), which was isolated and identified from the curd by 16 S rRNA sequencing. The probiotic properties of the isolated strain were studied by bile and NaCl tolerance and the ethyl acetate extract of *Ea-LpRD1*, was used to determine the toxicity against human breast cancer (MCF-7) cell lines and human embryonic kidney (HEK-293) cell lines by MTT assay. DNA fragmentation assay was carried out to study apoptosis induction. Flow cytometry analysis was done to determine the % of a cell population using the FTIC-Annexin V staining method. RT-PCR was used to assess gene expression levels in both cell lines. The IC50 concentration of the *Ea-LpRD1* in MCF-7 cells was 0.30 mg/ml and in HEK-293 was 0.47 mg/ml. The expression levels of the BCL-2 gene anti-apoptotic genes in humans were reduced and BAX, caspase-8, caspase-3, and caspase-9 were an increased expression in MCF-7 cell lines.

**Keywords** Breast cancer · Probiotics · *L plantarum* · Bcl-2, Bax · Caspases

**Introduction**

Cancers are distinguished by the fact that the cells expand beyond their normal growth limits, infect the surrounding cells, and spread to the organs. Males are more likely to develop lung, prostate, colorectal, stomach, and liver cancers, whereas females are more likely to develop breast, colorectal, lung, cervical, and gastric cancers (Sentürk et al. 2020). Women worldwide continue to suffer from breast cancer as a significant health hazard and death is only secondary to that of lung cancer. The annual incidence of new breast cancer cases has not decreased much during the previous few decades (Shen et al. 2020). As a result, research into innovative and effective techniques for treating breast cancer is in great demand. Probiotics are now being proposed as an adjuvant and supplementary method for improving the efficacy of chemotherapy and immunotherapy. Probiotics, interestingly, have distinct impacts on normal and malignant cells. The majority of probiotic bacteria are lactic acid bacteria (LAB), such as *Bifidobacterium* and *Lactiplantibacillus*. (Argyri et al. 2013). Probiotics are best delivered to customers via dairy products since they are the most convenient and appropriate carriers. (Yu et al. 2019). *Lactiplantibacillus plantarum* also called *Lactobacillus plantarum* is a promising option in probiotics for cancer treatment (Asoudeh-Fard et al. 2017). Lactic acid bacteria (LAB) are a diverse group of Gram-positive, acid-tolerant, non-sporulating rod or cocci microorganisms, and they play an important role in the fermentation of food processing. Many other sources, including milk products, fermented foods, animal intestines or fresh-water fish, soil samples, sugar cane plants, and poultry farms, could be used to isolate LAB (Mulaw et al. 2019).

Curd is made by the fermentation of cow or buffalo milk typically utilizing the previously preserved curd as a starter in most families, and it is an important component of the daily meal in most households. (Sunil Kumar et al. 2018)
The probiotic bacteria which was isolated from the curd have been characterized for probiotic properties such as acid tolerance, and bile tolerance it also has many health benefits. The earlier reports suggest that it also exhibits an anti-tumor effect in both animals and humans (Górńska et al. 2019). Apoptosis is a genetically programmed cell death that enables the removal of cells that are genetically harmed, have lost their function, develop in an irregular manner, or are overcrowded. Apoptosis is essential for the development of normal breast cells. Apoptosis rates are connected to tumor grade, with more aggressive tumors having greater apoptosis and proliferation rates. Most of the control point pathways are overridden when the apoptotic process is disrupted, resulting in the growth of neoplastic cells. Apoptosis-controlling genes and proteins can be used as modification targets to enhance cancer cell death. As a result, apoptosis regulation is critical in the therapy of cancer (Wong, 2011). B-cell lymphoma-2 protein (Bcl-2) is a critical regulator in the apoptosis molecular processes (Chipuk et al. 2010). When linked with the development of a Bax/Bax homodimer, this family of proteins functions as a Bcl-2-associated protein X (Bax) promoter, which causes and accelerates cell death. Bax inhibits the production of Bcl-2 and B-cell lymphoma extra-large (Bcl-xL) anti-apoptotic proteins by forming Bcl-2/Bax or Bcl-xL/Bax heterodimers. The fate of the cell is determined by complex networks of interactions between members of the Bcl-2 family, both cytosolic and mitochondrial (Lindsay et al. 2011). The caspase family is a collection of cysteine proteases that trigger apoptosis in two pathways. Death receptors are required for the first pathway (Extrinsic), whereas mitochondria are required for the second pathway (Intrinsic). The activation of caspases is linked to the induction of both apoptotic pathways. The primary death receptor signaling caspase is caspase-8 (Wieder et al. 2001). Effector caspase-3 is a death protease that catalyzes the particular cleavage of a variety of important cellular proteins. Caspase-3 activation mechanisms have been discovered that are both reliant and independent of mitochondrial cytochrome c release and caspase-9 activation (Yang et al. 2007). Caspase-3 is essential for apoptotic chromatin condensation and DNA fragmentation, and it is the hallmark of apoptosis. As a result, caspase-3 is required for the processes of cell rupture and the creation of apoptotic bodies. Caspase-8 is an initiating caspase that is activated by death receptor stimulation, but it is also needed by other apoptotic triggers (Krüdinger and Evan 2000).

Although this microorganism’s therapeutic potential has been discovered, the molecular pathways by which it induces anti-cancer effect(s) are yet unknown. Probiotics are thought to have the ability to alter cell signaling pathways. The major goal of this study was to figure out the molecular mechanism(s) of probiotics to fight against cancer. Thus, in the present investigation, the cytotoxic effect of ethyl acetate extract of Lactiplantibacillus plantarum RD1 (Ea-LpRD1), which was isolated from curd, against normal and breast cancer cells was demonstrated. The molecular mechanism of the extract toward the breast cancer cell lines was studied using gene profiles that were involved in inducing apoptosis. A very few studies were conducted on the apoptotic gene regulation using probiotics.

Materials and methods

Isolation and identification of bacterial strain

In this research, homemade curd was used (as a sample) for the isolation of Lactobacillus (LAB). Curd samples were obtained from an urban area home, Tirupati, Andhra Pradesh, India. Lactobacilli were isolated from curd using the conventional serial dilution and selective MRS (Man, Rogosa & Sharpe) agar media (Himedia, GM641) plating was incubated at 37 °C for 48 h. The bacterial colonies of Lactobacilli were purified using subcultures. The obtained single colony were collected, inoculated in 15 ml of MRS broth and was incubated at 37 °C for 48 h. To identify the isolate, Gram’s staining and catalase tests were performed. For subsequent testing, the isolates were kept at − 80 °C in 30 percent (w/v) glycerol with MRS broth (Patil et al. 2010).

Molecular characterization

Isolation of DNA from Lactobacillus bacterial strain

Total genomic DNA was isolated from a 3 ml culture of each pure colony maintained overnight at 37 °C in MRS broth. With slight changes, the DNA was isolated as reported in (Sarma 2019). The cultures were centrifuged for 3 min at 10,000 rpm at 4 °C, with the supernatant discarded. The cells were then resuspended by pipetting and incubated for 5 min at room temperature in solution I (50 mM Tris–HCl buffer, pH 8.0, 50 mM EDTA, and 20% glucose). After that, 0.1 ml of 10 mg/ml lysozyme was added and incubated at 37 °C for one hour. To the above solution, about 0.03 ml of RNase buffer was added and incubated at 37 °C for 30 min. The 0.1 ml of 10% SDS was added and mixed well and incubated for 30 min at 37 °C. The equal volumes of solution II (Chloroform:Isoamyl alcohol in 24:1 (v/v)) were added and mixed vigorously and centrifuged at 10,000 rpm for 15 min at 4 °C. To the aqueous layer, an equal volume of ice-cold isopropanol was added and mixed by vortexing, and the same was centrifuged then 0.5 ml of 70% ethanol was mixed and centrifuged at 10,000 rpm for 10 min at 4 °C.
The pellet obtained was air-dried and dissolved in 0.05 ml of TE buffer (10 mM Tris–HCl pH 8.0 and 1 mM EDTA). The DNA obtained was analyzed by running 1% agarose gel electrophoresis.

16S rRNA gene sequencing

A portion of the 16S rRNA gene was amplified by PCR using genomic DNA as a template. The two universal primers utilized in this investigation were 27 F (5ʹ-AGA GTT TGA TCC TGG CTC AG-3ʹ) and 1492 R (5ʹ-GGT TAC CTT GTT ACG ACT T-3ʹ) (Weisburg et al. 1991). A single band reflecting the amplified 16S rRNA gene product was obtained by PCR amplification. 1% agarose electrophoresis was used to analyze the PCR results, which were photographed with a UV Transilluminator. The purified DNA was sequenced with the help of an ABI 3730 x1 Genetic analyzer using the BDT v3.1 Cycle sequencing kit method from ThermoFisher.

Phylogenetic analysis

A consensus sequence of the 16S rRNA gene was produced from forward and reverse sequencing data. The 16S rRNA gene sequence was used to perform BLAST searches against the NCBI GenBank database. The first ten sequences were chosen and aligned using Clustal W, a multiple alignment software tool, based on their maximum identity score. MEGA-X was used to create the distance matrix and the phylogenetic tree (Kumar et al. 2018).

Characterization of probiotic properties

NaCl and bile tolerance

The tolerance of LAB isolate against various NaCl concentrations 2%, 4%, 6%, and 8% (w/v) was investigated. Test tubes containing 5 ml of MRS broth with 0.05 ml of overnight Lp culture with different concentrations of NaCl and incubated for 24–72 h at 37 °C. The OD was taken at 600 nm (Chowdhury 2012) against the relevant blank with the presence of growth as positive control and no growth as the negative control.

The bile salt tolerance was evaluated using the technique published by (Goel et al. 2020). 5 ml of mid-log phase isolate was supplemented with bile salt (Sigma–Aldrich, INDIA) concentrations (0.3%, 0.5%, 1.0%, 2.0%, 4.0%), and incubated at 37 °C. After 24 h of incubation at 37 °C, the bacterial growth was assessed. The % survival of the isolate was determined using a spectrophotometer at a wavelength of 560 nm (Chowdhury 2012). The blank with the presence of growth as positive control and no growth as the negative control.

Preparation of ethyl acetate crude extract of probiotic isolate EA-Lp

From the isolate, the crude cell-free extract was produced by growing the culture in 50 ml of MRS broth at 37 ± 2 °C for 24–72 h. Then the culture was centrifuged at 10,000 rpm for 10 min at 4 °C. For the supernatant: 2:1 ratio of ethyl acetate was used. By mixing it vigorously in the separating funnel and leave it for 5–10 min. Two separating layers were found. From these layers, the organic layer was collected and kept in a rotary evaporator to obtain the bioactive compounds from it. The obtained solution was filtered using a 0.22 μm syringe filter and stored at −20 °C for further use (Chakraborty et al. 2022).

Anticancer activity

Cell lines maintenance

The human adenocarcinoma (MCF-7) and human embryonic kidney (HEK-293) cells used in this study were procured from National Centre for Cell Science (NCCS), Pune, INDIA. The cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM HG Himedia, AT007) with 10% Fetal Bovine Serum (FBS, Himedia RM10432) 50 µg/ml of penicillin and streptomycin antibiotic solution and incubated at 37 °C and 5% CO2 in a CO2 incubator and were subcultured for every 2 days for routine maintenance.

MTT assay

For the cytotoxicity experiment, cells (2 × 10⁴/well) were seeded in a 96-well plate and incubated for 24 h at 37 and 5% CO2. After 80% confluency the cells were treated with the different concentrations (25, 50, 100, 200, 400 µg/ml) of Ea-LpRD1. After 24 h of incubation, 0.02 ml of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), (Himedia RM1131) (5 mg/ml in PBS) was added to each well and incubated for 3 h in a CO2 incubator. The MTT solution from each well was removed and to solubilize the formazan crystals, 0.1 ml of dimethyl sulphoxide (DMSO, Sigma S-002-D) was added to each well. At 570 nm, crystals and absorbance were measured on a microplate reader. Camptothecin (12.5 µM) with the medium was taken as positive control and the media without experimental compound is taken as negative control (Chakraborty et al. 2021). The IC₅₀ value was determined using the logarithmic regression equation Y = Mx + C or Y = Mlogx + C. The percentage of cell viability is calculated using the following formula:

\[
\% \text{ Cell viability} = \left( \frac{\text{Mean OD of Sample at } 570 \text{nm}}{\text{Mean OD of Untreated at } 570 \text{nm}} \right) \times 100.
\]
DNA fragmentation analyses

The Ea-LpRD1 was tested for its apoptotic potential by DNA fragmentation analysis. The MCF-7 cells (2 × 10^4 cells) were seeded in a six-well plate along with the media and incubated for 24 h in a CO₂ incubator. For the genomic DNA isolation, the cells were treated with IC₅₀ value was obtained based on MTT assay and then they were centrifuged. The pelleted cells were centrifuged with 0.5 ml of ice-cold lysis buffer (20 mM Tris–HCl, pH 7.4, 10 mM EDTA, and 0.5 percent Triton X-100). RNase A (10 µg/ml) was added to the supernatant and incubated for one hour at 37 °C, followed by protease K (20 µg/ml) for incubation of 2 h at 6 °C. Pure phenol was added in equal volumes and gently agitated for 30 min and centrifuged at 14,000 rpm for 5 min. The aqueous layer was collected the equal volume of (1:1) concentration of phenol and chloroform was added and again centrifuged.

The top phase was collected and DNA was precipitated using twice as much as absolute alcohol and centrifuged at 10,000 rpm for 4 min. The obtained pellet was washed with 70% ethanol and air-dried and suspended in 50 µl of TE buffer. The DNA was resolved electrophoretically on 1.8 percent agarose gel in 1 × TAE buffer (40 mM Tris–acetate, 1 mM EDTA) and stained with ethidium bromide (0.5 g/ml) under a UV trans-illuminator. (Green and Sambrook 2012)

MCF-7 cells with a cell concentration of (2 × 10⁴ cells) with Ea-LpRD1 was taken as test and untreated MCF-7 cells were taken as control (Mojibi et al. 2019).

Flow cytometry analysis to determine the cell apoptotic and necrotic induction in MCF-7 cells

To determine the cell apoptosis and necrosis after Ea-LpRD1 treatment, the flow cytometry (BD FACSCalibur) analysis was performed using FITC Annexin V (51-65874X, BD Biosciences). In six-well plate, the MCF-7 cells (2 × 10^5 cells/2 ml) were incubated in CO₂ incubator at 37 °C for 24 h. Then the cells were treated with the IC₅₀ concentration (301.8 µg/ml). After incubation the monolayer was washed with PBS and 200 µl of trypsin–EDTA was added and incubated for 3–4 min then centrifuge at 500 rpm for 5 min at 25 °C. The supernatant was discarded and the cells were washed with PBS. To the cells, 5 µl of FITC Annexin V was added. The cells were vortexed and incubated at room temperature for 15 min. To this, 5 µl of propidium iodide (51-66211E, BD Biosciences) and 400 µl of 1× binding buffer were added to each tube and vortexed gently. The cytometry analysis was performed using the untreated cell line as the negative control. The obtained data were analyzed using a BD CellQuest pro VER 0.6.0. Software (Baghbani-arani et al. 2019).

RNA isolation and real-time reverse transcription PCR

The MCF-7 cells (2 × 10⁵ cells) were seeded in a six-well plate and incubated overnight to get attached and the cells were treated with the Ea-LpRD1 with IC₅₀ concentration (301.8 µg/ml) and the untreated cells are considered as a negative control. The total RNA was isolated from both treated and untreated cells using the MEDOX RNA isolation kit (Medox Biotech, India). The obtained RNA was stored in a − 20 °C refrigerator and 2 µg of the total RNA was used for cDNA conversion using the cDNA reverse transcription kit method (Applied biosystems). The expression of genes in both untreated and treated cells was analyzed by real-time PCR. The specific primers used in this study are listed in Table 2 and were purchased from (Eurofins and Macrogen). SYBR green mix (10 µl) reaction volume was used in the RT-PCR (AB PRISM 7300, Applied Biosystems). The relative changes in mRNA expression levels were analyzed by 2^-ΔΔCT.

Statistical analysis

All parameters were measured in triplicate, and the findings were reported as mean standard deviation (SD), using Graphpad Prism 9 software. MEGA-X software was used to create the phylogenetic tree.

Results and discussion

Identification of bacterial isolate from curd

In this study, more than 50 colonies were isolated. Only a few bacterial colonies were chosen for further investigation after morphological evaluation. Colonies were chosen based on their ability to be catalase-negative, which is a hallmark for lactobacillus bacteria. Gram staining was used for further identification and microscopic examination showed that the round, regular, whitish which was

Fig. 1 Bacterial colony isolated from homemade curd on MRS media
selected as sample. The sample was Gram-positive. The morphological, biochemical, and molecular characterization of isolated bacterial strains are shown in Fig. 1 and Table 1.

**Molecular characterization through 16S rRNA and phylogenetic analysis**

16S rRNA gene sequencing and phylogenetic analysis were used to identify the isolates at the sub-species level. Isolated bacterial 16S rRNA genes (500 bp) were amplified and sequenced using PCR as shown in Fig. 2a. The obtained sequences were compared with the lactic acid bacterial strains in the Genbank database. BLASTn was used to identify the isolated bacteria as *Lactiplantibacillus* (*Lactobacillus plantarum*) strains. The nucleotide sequence of *Lactiplantibacillus Plantarum* RD1 was deposited in NCBI Genbank and the accession number allotted is shown in Table 1. The BLAST analysis result was subjected to MEGA-X programme to determine the closely related species. These results were found to be identical to *Lactobacillus plantarum* SN13T, and the phylogenetic tree of LpRD1 is shown in Fig. 2b.

**Isolated strain’s probiotic properties**

**NaCl tolerance**

To determine the impact of salt tolerance and the growth of isolate from the curd (*Lpb RD1*), the growth media (MRS broth) was added with 2, 4, 6, 8, 10% (w/v) NaCl. As NaCl is an inhibitory chemical, it may inhibit the growth of certain bacteria. Probiotics must withstand high at salt concentrations in the human gut. The results demonstrated that the *Lactiplantibacillus plantarum* species isolated from curd

---

**Table 1** The morphological, biochemical, and molecular characterization of the isolate

| Isolate | Colony shape | Colony margin | Colony color | Catalase | Gram’s staining | Stain name | 16S rRNA amplified gene length | Assession number |
|---------|--------------|---------------|--------------|----------|----------------|------------|-----------------------------|-----------------|
| Sample 1 | Round        | Regular       | Whitish      | Negative | Positive       | *Lactiplantibacillus plantarum* RD1 | 1496 bp         | MZ476213         |

---

**Fig. 2** A 16S rRNA amplicon of isolate with 1KB ladder. B Phylogenetic tree showing the isolate MZ476213
there was a rapid impact on cell viability at 400 µg/ml in MCF-7 cells whereas in HEK-293 cells the extract failed to show potential toxicity. The cytotoxic potential of Ea-Lp RD1 against MCF-7 and HEK-293 cell lines are shown in Fig. 6. From the results, it was concluded that the extract is more toxic to breast cancer cell lines when compared to normal cell lines. The IC50 values of both MCF-7 and HEK-293 cells are 301.87 µg/ml and 471.55 µg/ml are shown in Fig. 5b, c.

DNA fragmentation of Ea-LpRD1 treated with MCF-7 cells

To determine the apoptotic potential of Ea-LpRD1, DNA fragmentation was carried out. Mcf-7 cells were treated with IC50 concentration (301.8 µg/ml) of Ea-LpRD1. After 24 h, the presence of oligonucleosomal DNA fragments was observed, on 1.8% Agarose gel electrophoresis as shown in Fig. 7. which suggests the induction of apoptosis in MCF-7 cell lines.

Flow cytometry analysis of Ea-LpRD1 extract against MCF-7 cells

To check the apoptotic nature of the Ea-LpRD1 extract against MCF-7 cells, flow cytometry analysis was performed using FITC Annexin V staining. As shown in Fig. 8, the MCF-7 cells were treated with the IC50 concentration of Ea-LpRD1 extract and after 24 h incubation, 44.96% of early apoptosis and 25.53% of late apoptosis were observed when compared with the untreated. Figure 8a, d FSC represents the forward scatter SSC represents side scatter of Ea-LpRD1 against MCF-7 cells and Fig. 8b reveals the fluorescent dot blots reveals as a viable population in the lower left quadrant, whereas in Fig. 8e the fluorescent dot blots are in the middle which represents early apoptosis, late apoptosis, and viability. Figure. 8c and f represents the effect of cell cycle progression with cell cycle arrest. Figure 8g reveals a graph that represents the percentage of live cells, early apoptotic, late apoptotic, and necrosis. From the results, it was revealed that the cells treated with Ea-LpRD1 extract are showing toxic effects on breast cancer (MCF-7 cell lines) through induction of apoptosis (Rudrappa et al. 2022).

RT-PCR

Caspase family activation has been identified as one of the earliest known steps in the cell death process. Caspase-3 activation is involved in the regulation of both intrinsic and extrinsic apoptotic pathways. Bcl-2 belongs to a wide family of cell survival controlling proteins that includes both pro- and anti-apoptotic regulators. Cancers use the Bax/Bcl-2 pathway as their primary apoptosis evasion mechanism. As a result, we evaluated the activity of the genes Bax, Bcl-2,
Fig. 5  A The cytotoxic activity *Ea-LpRD1* against MCF-7 and HEK-293 cells. B The graph representing the IC50 value of *Ea-LpRD1* against MCF-7 cells and C The graph representing the IC50 value of *Ea-Lp RD1* against HEK-293 cells

![Graphs showing cytotoxic activity](image)

Fig. 6  The cytotoxic activity *Ea-LpRD1* of against MCF-7 and HEK-293 cells: a untreated, b Standard, c 25 µg/ml, d 50 µg/ml, e 100 µg/ml, f 200 µg/ml, and g 400 µg/ml
caspase-3, -8, and -9. Our results indicates that the *Ea-Lp* RD1 treated with MCF-7 cells was showing increasing levels in apoptotic genes. As shown in Table 2 the listed apoptotic primers were synthesized and used in this study. The relative mRNA expression of the apoptotic genes is shown in Fig. 9. The GAPDH gene is used as an internal reference. The expression of Bcl-2 was decreased and the other genes such as Bax, caspase-3, caspase-9, and caspase-8 have increased in their expression levels (Alotaibi et al. 2021).

### Discussion

Although cancer treatments have recently improved, malignancies remain the second-largest cause of death globally. Probiotics were recommended to increase

---

**Fig. 7** DNA fragmentation analysis of *Ea-Lp* RD1 treated with MCF-7 cells. **A** 1 Kb Ladder fragmented DNA, **B** Control, **C** MCF-7 cells treated with *Ea-Lp* RD1 at (301.8 µg/ml)

**Fig. 8** Flow cytometric analysis of cells treated with *Ea-Lp* RD1 against MCF-7 cells. **a** Untreated, **b** Untreated Q1 shows the necrosis and cell debris, Q2 shows the late apoptosis, Q3 shows viable cells and Q4 shows the early apoptosis. **c** Untreated, **d** *Ea-Lp* RD1-treated cells, **e** *Ea-Lp* RD1-treated cells Q1 shows the necrosis and cell debris, Q2 shows the late apoptosis, Q3 shows viable cells and Q4 shows the early apoptosis. **f** *Ea-Lp* RD1-treated cells, **g** Graph representing the % of cell population showing the early apoptosis, late apoptosis, viability, and necrosis.
the effectiveness of anti-cancer drugs as additional and complementary treatments. But it is still impossible to completely understand the molecular mechanisms of the actions of probiotics on cancer cells (Asoudeh-Fard et al. 2017). Chemotherapy, radiotherapy, and surgery are the most frequent treatments, although they can harm normal cells as well as malignant cells. As a result, several research projects in this field have been conducted. Hence, we isolated a novel Lactiplantibacillus plantarum RD1 from the homemade curd, and its potential was evaluated by testing the obtained probiotic at high acidic and bile salt concentrations. (Huang et al. 2015) suggested that the L. plantarum ZDY 2013 isolated from acid beans, China has shown better survival at high concentrations of acid and bile salt. Our findings suggest that Lactiplantibacillus plantarum RD1 also showed the best survival compared to the other reports. Luang-In et al. 2020 revealed that the probiotic isolated from a fermented vegetable product from Thailand (Pak-Sian Dong) Lactobacillus Plantarum KK518 showed more efficacy towards MCF-7 cells compared to the previous report (Chuah et al. 2019) findings that six strains were isolated and identified from bacteriocin-producing L plantarum produced postbiotic metabolites (from Malaysian foods) were more cytotoxic towards cancer cells.

Asoudeh-Fard et al. 2017 suggests that L plantarum induces apoptosis in oral cancer cells by upregulation of PTEN and downregulation of MAPK signaling pathways. The induction of apoptosis was confirmed using an Annexin V-FITC-PI apoptosis detection test, which is widely used to distinguish living cells from both early and late apoptosis. Our results are similar to Rudrappa et al. 2022, they used synthesized P-AgNPs against U118 MG cells, which resulted in the 42.2% of apoptosis and 3.41% of necrosis. Similar results of DNA fragmentation and flow cytometry analysis were observed in (RiazRajoka et al. 2019) which suggests that the cell-free supernatant of the probiotic that is isolated from human breast milk showed an increase and decrease in the expression levels of mitochondria-mediated apoptosis pathway genes (BAX, BAD, Bcl-2, caspase-3, caspase-8, and caspase-9 genes) in cervical cancer. Similarly, the results of the present study also showed the potential effect anti-cancer effect on breast cancer cells and also showed significant results in the expression levels of mitochondria-mediated apoptotic genes. The similar results were observed in (Alotaibi et al. 2021), where the Saussurea lappa was treated with Hep2 cell line.

**Conclusion**

In the present study, the isolated Lactobacillus spp. meets the probiotic criteria, including tolerance to adverse circumstances such as high salt, low pH, and high bile salt concentration. The selective cytotoxic effect of the Ea-LpRD1 against MCF-7 breast cancer cell lines was

---

**Table 2** The list of primers used for RT-PCR

| GENE    | Forward   | Reverse               |
|---------|-----------|-----------------------|
| Caspase-3 | CAGCCAGGAGAAAATCAACAGA | TTGGCACCTTTCCGTTAACC   |
| Caspase-9 | TTGCCACCTTTCCGTTAACC   | TTGCCACCTTTCCGTTAACC   |
| Caspase-8 | GAGATGGAAGAGGAACCTTACAGA | AGCATGACCCGTAGGACAGAA  |
| Gapdh   | GCCATCCTGCGCTACACTGA    | GAGTGGTGTCGCGCTTGGAAA  |
| Bcl-2   | TCCGGGAGGAAGTCCAAATG    | CAGCCAGGGAATCAACAGA    |
| Bax     | ACCAAGGTGCCGGAACAG     | TCCGGAGGAAGTCCAAATG    |

**Fig. 9** The relative mRNA expression of apoptotic genes treated with Ea-Lp RD1 against MCF-7 cells.
demonstrated. The extract showed a potential effect on MCF-7 cancer cell lines when compared to HEK-293 cell lines, taken as normal cell line models. In conclusion, we report that the apoptotic induction in the MCF-7 cell line through the negative regulation of the Bcl-2 gene and positive regulation of Bax, caspase-3, caspase-8, and caspase-9 genes by Ea-LpRD1. As a result, it was proposed that Ea-LpRD1 could be employed in the treatment of breast cancer following additional in-vivo investigations. Future research is needed to completely understand the mechanism involved in vivo assessment of the anti-cancer efficacy.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-019-01679-8.

Acknowledgements R. Durga is highly thankful to DST (Department of Science and Technology), Govt. India, New Delhi in the form of DST-INSPIRE FELLOWSHIP for providing financial support to pursue PhD programme (No.DST/INSPIRE Fellowship/2016/IF160967).

Author contributions DR carried out all the experiments and manuscript was prepared with the help of SKS PGVK and SY designed and supervised the project and helped in manuscript drafting.

Funding This work was supported by DST, Govt. India, New Delhi in the form of DST-INSPIRE FELLOWSHIP for providing financial support to pursue PhD programme (No.DST/INSPIRE Fellowship/2016/IF160967).

Data availability The genome data of Lactiplantibacillus plantarum RD1 16S ribosomal RNA gene, partial sequence are available through GenBank accession MZ476213, respectively.

Declarations

Conflict of interest We declare no conflicts of interest.

References

Alotaibi A, Asmatanzeem B, Rasha ASSADA, Shaik KN, Sreenivasa N, Muthuraj R, Shashiraj KN, Meghashyama PB (2021) Saussurea Lappa exhibits anti-oncogenic effect in hepatocellular carcinoma, Hepg2 cancer cell line by Bcl-2 mediated apoptotic pathway and mitochondrial cytochrome c release. Current Issues Mol Biol 43(2):1114–1132

Argyri AA, Zoumpopoulou G, Kartzas K (2013) Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food Microbiol 33(2):282–291

Asodeh-Fard A, Abolfazl Barzegari AD, Sepideh B, Asal G, Yadollah O (2017) Lactobacillus plantarum induces apoptosis in oral cancer kb cells through upregulation of PTEN and downregulation of MAPK signaling pathways. Biomed Research International 7(3):193–198. https://doi.org/10.15171/bri.2017.22

Baghbani-arani F, Asgavy V, Hashemi A (2019) cell-free extracts of lactobacillus acidophilus and lactobacillus delbrueckii display antiproliferative and antioxidant activities against HT-29 Cell Line. Nutr Cancer 72(8):1–10. https://doi.org/10.1080/0163581.2019.1685674

Chakraborty B, Raju SK, Adブルラハマ RA, MuthurajRudrappa DK, Pallavi SS, Halaswamy H, Karthikeyan P, Sreenivasa N (2021) Evaluation of antioxidant, antimicrobial and antiproliferative activity of silver nanoparticles derived from galphimia glauca leaf extract. J King Saud Univ Sci 33(8):101660. https://doi.org/10.1016/j.ksus.2021.101660

Chakraborty Raju B, Abraham SKIA, Pethaiah G, Sreenivasa N (2022) Bioprospection and secondary metabolites profiling of marine streptomyces levis strain KS46. Saudi J Biol Sci 29(2):667–679

Chipuk J, TUDor M, Fabien Parson LMI, Douglas Green R (2010) Review the BCL-2 family reunion. Mol Cell 37(3):299–310. https://doi.org/10.1016/j.molcel.2010.01.025

Chowdhury A (2012) Screening of Lactobacillus spp. from buffalo yoghurt for probiotic and antibacterial activity. J Bacteriol Parasit Vol 03(08):1–6

Chuah L, Foo H, Loh T, Alithseen N, Yeap S, Nur Elina A, Mutahil R, Rahim and Khatijah Yusoff, (2019) Postbiotic metabolites produced by lactobacillus plantarum strains exert selective cytotoxicity effects on cancer cells. BMC Complement Altern Med. https://doi.org/10.1186/s12906-019-2528-2

Goel A, Halami PM, Tamang JP (2020) Genome analysis of Lactobacillus Plantarum isolated from some Indian fermented foods for bacteriocin production and probiotic marker genes. Front Microbiol 11(1January):1–12

Górska A, Przy stumpski D, Niemczura MJ, Kulbacka J (2019) Probiotic bacteria a promising tool in cancer prevention and therapy. Curr Microbiol 76(8):939–949. https://doi.org/10.1007/s00284-019-01679-8

Green MR Sabbathoom J (2012) Molecular Cloning. A Laboratory Manual vol 1, 4th Edition https://www.cshlp.com/pdf/sample/2013/MC4/MC4FM.pdf

Huang R, Tao CuiXiang Xi, Li WS, Xu H, Xu F, Nagendra P, Shabh-Hua W (2015) In vitro probiotic characteristics of Lactobacillus Plantarum ZDY 2013 and Its modulatory effect on gut microbiota of mice. J Dairy Sci 98(9):5850–5861. https://doi.org/10.3168/jds.2014-9153

Kruidering M, Evan GI (2000) Caspase-8 in apoptosis the beginning of the end? IUBMB Life 50(2):85–90

Kumar Stecher SG, Li Knyaz MC, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549

Lindsay J, Degli M, Gilmore AP (2011) Biochimica et biophysica acta Bcl-2 Proteins and Mitochondria—Specificity in membrane targeting for death. BBA—Mol Cell Res 1813(4):532–539. https://doi.org/10.1016/j.bbamcr.2010.01.017

Luang-In V, Saengha W, Buranrat B, Nudnamud-Thanoi S, Narbad A, Pumriw S, Samappito W (2020) Cytotoxicity of Lactobacillus Plantarum KK518 isolated from Pak-Sian Dong (Thai fermented Gynandropsis pentaphylla DC.) against HepG2, MCF-7 and HeLa cancer cells. Pharmacognosy J 12(5):1050–1057

Mojibi P, Tafvizi F, Torbati MB (2019) Cell-bound exopolysaccharide extract from indigenous probiotic bacteria induce apoptosis in HT-29 Cell-Line. Iran J Pathol 14(1):41–51

Mulaw G, Tessema TS, Muleta D, Tessema A (2019) In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented ethiopian food products. Intern J Microbiol 5(3):13

Patil MM, Ajay Pal Anand T, Ramana KV (2010) Isolation and characterization of lactic acid bacteria from curd and cucumber. Indian J Biotechnol 9(2):166–172

Riaz Rajoka MS, Zhao H, Mehwish HM, Li Na, Yao Lu, Lian Z, Shao D, Jin M, Li Q, Zhao L, Shi J (2019) Anti-tumor potential of cell free culture supernatant of Lactobacillus rhamnosus strains isolated from human breast milk. Food Res Int 123(May):286–297
Rudrappa M, Rudayni HA, Assiri RA, Bepari A, Basavarajappa DS, Nagaraja SK, Chakraborty B, Swamy PS, Agadi SN, Niazi SK, Nayaka S (2022) Plumeria alba-mediated green synthesis of silver nanoparticles exhibits antimicrobial effect and anti-oncogenic activity against glioblastoma u118 mg cancer cell Line. Nanomaterials 12(3):493

Sarma, P.V.G.K. 2019. MOLECULAR BIOLOGY. A Practical Manual MJP Publisher.https://books.google.co.in/books?id=LoybDwAAQBAJ

Sentürk M, Ercan F, Yalcin S (2020) The secondary metabolites produced by Lactobacillus Plantarum downregulate BCL-2 and BUFFY genes on breast cancer cell line and model organism drosophila melanogaster: molecular docking approach. Cancer Chemother Pharmacol 85(1):33–45. https://doi.org/10.1007/s00280-019-03978-0

Shen F, Pan X, Li M, Chen Y, Jiang Y, He J (2020) Pharmacological inhibition of necroptosis promotes human breast cancer cell proliferation and metastasis. Onco Targets Ther 13:3165–3176

Devaraja G et al (2018) Screening of Probiotic Potential of Lactobacillus Strains Isolated using Homemade Curd from Mysore District, Karnataka. EC Microbiology 14(7):374–383

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173(2):697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Wieder T, Essmann F, Prokop A, Schulze-oshoff K, Bevaert R, Dorken B, Daniel PT (2001) “Activation of caspase-8 in drug-induced apoptosis of b-lymphoid cells is independent of CD95 / Fas receptor-ligand interaction and occurs downstream of caspase-3. Blood 97(5):1378–1387

Wong RSY (2011) Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res 30(1):1–14

Yang S, Zhou Q, Yang X (2007) “Caspase-3 status is a determinant of the differential responses to genistein between MDA-MB-231 and MCF-7 breast cancer cells. BBA Mol Cell Res 1773:903–911

Yu H-S, Lee N, Choi A, Choe J, Chun-Ho H, Bae, Paik (2019) Antagonistic and antioxidant effect of probiotic Weissella cibaria JW15. Food Sci Biotechnol 28(3):851–855. https://doi.org/10.1007/s10068-018-0519-6

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.