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Antimicrobial Activities of Lipoxazolidinone A Purified from *Lactobacillus apis* YMP3 against Various Infant Diarrheal Pathogens and its Cytotoxicity against Normal Mammalian Cells

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Infant diarrheal infections continue as one of the critical challenges of global health issues, demanding the development of safe therapeutic solutions; hence, this study was performed. This study explores the antimicrobial potential of lipoxazolidinone A against various infant diarrheal infectious pathogens and its cytotoxicity against mammalian cells. As reported earlier in our studies, this compound was produced and purified from a probiotic *Lactobacillus apis* YMP3. The antimicrobial study was performed in this study against a panel of 12 clinical bacterial pathogens causing infant diarrheal infections, collected from the Thanjavur Government Medical College, Thanjavur, Tamil Nadu, India. The purified lipoxazolidinone A showed appreciable antimicrobial activities against seven pathogens viz., *Vibrio cholerae, Proteus mirabilis*, *Salmonella typhi, Vibrio vulnificus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella paratyphi*, at the minimum inhibitory concentration of 100, 125, 150, 150, 150, 175, and 200 µg/ml, respectively, and the remaining five pathogens, viz., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Shigella dysenteriae*, and *Shigella boydii*, revealed partial growth inhibition of 73, 56, 37, 27 and 21% at the highest tested concentration (200 µg/ml), respectively. The cytotoxicity studies of lipoxazolidinone A using two normal mammalian cell line cultures, viz., primary mouse embryonic fibroblast cells (NIH/3T3) and Madin-Darby Canine kidney cells (MDCK), evidenced negligible toxicity till 400 µg/ml. These findings reveal that lipoxazolidinone A is a promising antimicrobial candidate, proving its appreciable inhibitions against pathogenic bacteria and nontoxicity towards mammalian cells. The mammalian cell-safe antimicrobial activities of lipoxazolidinone A suggest futuristic research for the potential drug development in treating many infant gastrointestinal infections.

Keywords: Bioactive compound, Clinical pathogens, Gastrointestinal infections, Mammalian cell safe, Probiotic strain

Introduction

Diarrheal diseases are the second leading cause of childhood death worldwide.[1] Diarrhoea causes over 1.3 million deaths annually among children under five years of age, presenting a significant global health challenge.[2] Various bacteria, fungi, viruses, and parasites commonly cause these infant gastrointestinal infections.[3] Among them, bacterial pathogens are some of the most common causes of infant diarrhoea, especially the genus of *Salmonella*, *Vibrio*, *Shigella*, *Pseudomonas*, *Escherichia coli*, etc.[4,5] Despite considerable improvements that have been made in developing countries since the last decade in the standard of living, sanitation, water treatments, and food safety awareness, diarrheal diseases continue to cause substantial economic and social losses.[2,6] In addition to the above, the rising concern over antimicrobial resistance signifies the identification of novel drugs for vulnerable populations, especially infants.[7,8]

Hence, the scientific advancements in therapeutics are now focused on reducing the mortality rates against these infant gastrointestinal infections.[9] Strains of *Lactobacillus* have gained significant interest in the field of food and biomedical due to their multiple roles in preserving food, enhancing human health, and their considerations on the GRAS status of microbes.[10] *Lactobacillus* strains, commonly isolated from fermented dairy products, are known to produce bioactive compounds, including hydrogen peroxide, organic acids, and bacteriocins, which show strong antimicrobial effects against many harmful microbes.[11] These metabolites play a significant role

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in maintaining gut health, competence in neutralising toxins, directly inhibiting the growth of pathogens, etc.[12,13] The mechanism of action of some antimicrobial metabolites produced by *Lactobacillus* strains was documented well beyond pH modification.[14]

For example, bacteriocin action against the growth of various pathogens and their non-toxic profile towards mammalian cells.[15,16] All these observations raise the appropriateness of metabolites derived from Lactobacillus strains in biomedical demand. In our earlier study, *Lactobacillus apis* YMP3 has been identified for synthesising a bioactive compound, lipoxazolidinone A, which has been reported for its potent antimicrobial activity, especially against *Vibrio cholerae*.[17,18] Lipoxazolidinone A features a distinctive 4-oxazolidinone core, complemented by an exocyclic conjugated ketone group and two lipophilic carbon chains.[19] To date, only a few reports have been available regarding its antimicrobial potential.[20–22] Considering the paucity of research, this study focused on the antimicrobial potential of purified lipoxazolidinone A against a panel of clinically isolated human pathogens from infant diarrheal patients. Additionally, the study examined its cytotoxic effects on normal mammalian cell line cultures, highlighting the compound's safe therapeutic uses.

Materials And Methods

Production and Purification of Lipoxazolidinone A

The production and purification of lipoxazolidinone A was performed from *Lactobacillus apis* YMP3, which was earlier isolated from yoghurt collected from the Thanjavur region, Tamil Nadu, India. This strain was molecular identified using 16S rRNA partial sequencing and the sequence was submitted in the NCBI GenBank database with the accession number, OM843103.1.(18) The strain is maintained on MRS agar (HiMedia, Cat. No. M641) slants at 4°C under refrigerated conditions and the synthesis of lipoxazolidinone A was confirmed in our previous study based on various spectral characterizations viz., FT-IR, NMR, GC and MS/MS analysis.[17] MRS broth (HiMedia, Cat. No. MV369) was used for the production medium of lipoxazolidinone A under standard cultural conditions of pH 6.5 and 35°C. After 72 hrs incubation, this compound was extracted from the cell-free broth with an equal volume of ethyl acetate, followed by HPLC purification using a reverse-phase C18 silica column as described earlier in our study.[17]

Antimicrobial Study of Lipoxazolidinone A

Pathogenic Strains

A panel of different infant diarrheal bacterial pathogens was used for the antimicrobial study of lipoxazolidinone A, they were *Salmonella typhi*, *Salmonella paratyphi*, *Vibrio cholerae*, *Vibrio vulnificus*, *Staphylococcus aureus*, *Shigella boydii*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Proteus mirabilis*, and *Klebsiella oxytoca*, respectively. These pathogenic strains were kindly gifted by Rajah Muthiah Medical College Hospital, Annamalai University, Tamil Nadu, India. These strains were maintained as axenic cultures in nutrient agar slants under 4°C refrigerated conditions. During the antimicrobial study, these strains were individually cultured on tryptic soy broth at 37°C, and the optical density of the exponential phase broth cultures was maintained at 0.1, which is equivalent to 10^8 CFU/mL inoculum concentration (according to 0.5 McFarland turbidity standard).[23]

Antimicrobial Assay

The antimicrobial activities of lipoxazolidinone A against the infant diarrheal bacterial pathogens were evaluated using the microdilution method.[24] For this assay, 24-well flat-bottom polystyrene microtiter plates with lids (Tarsons, India) were utilised. Well plates were filled with 125 µL of sterile double-strength tryptone soy broth, and 125 µL of the test sample solution was added to the corresponding wells. Subsequently, all the sample-containing well plates were individually tested for their antimicrobial efficacy. Different concentrations of purified lipoxazolidinone A were tested for their antimicrobial efficiency against all the individual pathogens. Additionally, a set of wells served as growth controls without any antimicrobial test samples, another set was supplemented with a known antibiotic, Linezolid (200 mg/L), as a reference standard drug which belongs to the same class of oxazolidinone antibiotics, and a separate set of wells had no inoculum or antimicrobial test/antibiotic compound, serving as a negative control. Except for the wells in the negative control set, all other wells were inoculated with 2.5 µL of the prepared individual pathogens at 10^8 CFU/ml inoculum concentration. After inoculation, the well plates were covered with lids and incubated

at 37°C for 48 hrs. Following incubation, the absorbance of the broth in each well was measured at 600 nm using a microplate reader (Biotek Elx808, WI, USA). The assays were performed in triplicate. The growth inhibition percentages of the tested bacterial strains were calculated according to the following formula:

$$\% \text{ Growth inhibition} = [(1 - (As/Ac)] \times 100$$

where, As represents the absorbance of the well with test samples and Ac represents the absorbance of the growth control well (without any added bioactive sample).

Confocal Laser Scanning Microscopy (CLSM)

CLSM was used to visualise the bacterial cell biomass in the microtiter plate assay method after antimicrobial treatment with different concentrations of lipoxazolidinone A. A loopful of broth culture was smeared on glass microscopic slides after treatment and fixed with 2% (v/v) glutaraldehyde in phosphate-buffered saline (PBS), pH 7.4 (137 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4) for 15 min. Excess fixative was removed by washing the smears with PBS for 15 min. The broth smears were then stained for bacterial cells with 0.01% (w/v) acridine orange (Sigma Chemicals, USA) in PBS for 15 min, which was followed by washing with PBS for 30 min to remove excess stain. The stained films were pictured in situ by CLSM with an Olympus LSMGB200 CLSM (Olympus Optical Co. Ltd., Tokyo, Japan). The CLSM with an argon ion laser at 488nm was used for excitation, and a 605–632 nm band-pass filter for emission. Images were captured using Olympus LSMGB200 CLSM bundled programs.[25]

Mammalian Cell Toxicity

Mammalian Cell Line Cultures

The cytotoxicity of lipoxazolidinone A was studied against the NIH/3T3 cell line derived from primary mouse embryonic fibroblast cells (ATCC No. CRL-1658) and Madin-Darby Canine Kidney (MDCK) derived from the normal kidney tissue of a normal adult female cocker spaniel (ATCC No. CCL-34), which were purchased from the American Type Culture Collection (ATCC), Rockville, MD, USA and maintained in Dulbecco's Modified Eagle Medium (DMEM). The cell line was cultured in a 25 cm^2 tissue culture flask with DMEM supplemented with sodium bicarbonate and 10% FBS and antibiotic solution containing: penicillin (100 U/ml) and streptomycin (100 µg/ml). The cultured cell line was kept at 37°C in a humidified 5% CO_2 incubator. Two-day-old confluent monolayer of cells was trypsinised, and the cells were suspended in 10% growth medium. 100 µl cell suspension (5×10^4 cells/well) was seeded in a 96-well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator for 24 hrs.

Cytotoxicity Assay

About five different concentrations of purified lipoxazolidinone A, viz., 50, 100, 200, 300, and 400 µg/ml, were supplemented with the 5% DMEM medium and were tested individually for cytotoxic activities. Briefly after 24 hrs of cell incubation, the growth medium was removed, and freshly prepared 5% DMEM medium containing the test samples was added to the respective wells and tested for cytotoxicity after 24 hrs of exposure. The viability of cells was evaluated by MTT assay.[26] Growth control was done using the same test procedure as mentioned above, with no added test sample. After incubation, the medium from the wells was carefully removed for the MTT (3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay. Each well was washed with 5% DMEM without FCS for 2-3 times, and 200µl of MTT (5 mg/mL) was added. The plate was incubated for 6 hrs in 5% CO_2 incubator for toxicity. After incubation, one ml of DMSO was added to each well, mixed, and left for 45sec. Viable cells present in the medium formed crystals, which were dissolved by adding a solubilising reagent, Dimethyl sulphoxide (DMSO), that resulted in the formation of a purple colour. The absorbance of the suspension was measured spectrophotometrically at 540 nm by taking DMSO as a blank.[26]

The percentage cell growth inhibition/cytotoxicity was calculated using the following formula,

$$\% \text{ Cytotoxicity} = 100 - [(At - Ab)/(Ac / Ab)] \times 100$$

The percentage cell growth was calculated using the following formula,

$$\% \text{ Cell growth} = [(At - Ab) / (Ac - Ab)] \times 100$$

where, At = Absorbance value of the test compound, Ab = Absorbance value of blank, and Ac = Absorbance value of the control

Microscopic Observation

Microscopic observations were also carried out for further confirmation after the treatment of mammalian

cells with lipoxazolidinone A. Calcein AM and Ethidium Homodimer III are fluorescent dyes utilised to stain the treated cells. Calcein AM is a membrane-permeable dye that stains viable cells, while Ethidium Homodimer III is a membrane-impermeable dye that stains dead cells. The staining was observed under a fluorescent microscope (ZEISS LSM 880, Germany). Fluorescence was recorded using a 490 nm excitation filter and a 520 nm emission filter for Calcein AM. For Ethidium homodimer III, a 545 nm excitation filter and a 620 nm emission filter were used. In the case of the combined dyes (Calcein AM and Ethidium homodimer III), a 495 nm excitation filter was applied, which is above the range of the 520 nm emission filter.

Results and Discussion

Antimicrobial Activity of Lipoxazolidinone A against Infant Diarrheal Pathogens

Lipoxazolidinone A is a bioactive compound first identified from a marine actinobacterium, *Marinispora* sp. NPS008920 procured from the sediments of Cocoa Lagoon, Guam[19] and initially reported for its antimicrobial activity against gram-positive pathogens. This structure was chemically known as 2-alkylidene-5-alkyl-4-oxazolidinones, which is similar to that of the commercial antibiotic linezolid (Zyvox), a 2-oxazolidinone.[27–29] To date, very few researchers have reported this structure; hence, very limited literature is available regarding its biological activities. In our previous study, we identified lipoxazolidinone A from a *L. apis* YMP3, which was the first report from a *Lactobacillus* species. Further, the purified lipoxazolidinone A has shown promising antimicrobial activity against the pathogenic bacterium *Vibrio cholerae.*[17] *To date, the antimicrobial activity of* Lipoxazolidinone A has been studied against a limited number of microbes, especially the strains of *Vibrio, Staphylococcus,* and *Enterococcus. On this backdrop, the* antimicrobial activity of the purified lipoxazolidinone A was studied against a panel of twelve infant diarrheal human pathogenic bacteria at different concentrations ranging from 50 to 200 µg/ml (Table 1). All the pathogenic strains showed growth inhibition against the purified lipoxazolidinone A, and the antimicrobial activity was concentration dependent which revealed a minimum sensitivity at the lowest tested concentration (50 µg/ml) and maximum inhibition at the highest tested concentration (200 µg/ml). Among the tested strains, complete growth inhibition was observed against seven of the twelve pathogens tested at various concentrations of lipoxazolidinone A.

These pathogens were *Vibrio cholerae, Proteus mirabilis*, *Salmonella typhi, Vibrio vulnificus, Bacillus cereus, Escherichia coli,* and *Salmonella paratyphi,* and the minimum inhibitory concentration was recorded at 100 µg/ml, 125 µg/ml, 150 µg/ml, 150 µg/ml, 150 µg/ml, 175 µg/ml, and 200 µg/ml, respectively. However, the standard reference antibiotic standard, Linezolid revealed moderate antimicrobial activity against *Salmonella typhi*, *Salmonella paratyphi and Proteus mirabilis.* Further, the remaining five pathogens showed partial growth inhibition against this lipoxazolidinone A. These pathogens were *Pseudomonas aeruginosa*,

Table 1 — Antimicrobial activities of Lipoxazolidinone A at various concentrations and the reference standard drug, Linezolid at 200 µg/ml, against a panel of twelve infant diarrheal human pathogenic bacteria; All experimental values are expressed as mean ± standard deviation (n = 3)

Human pathogens	Lipoxazolidinone A (µg/ml)							Linezolid (µg/ml)
	50	75	100	125	150	175	200	200
Salmonella typhi	35 ± 1.5	53 ± 1.9	71 ± 3.2	89 ± 4.1	100 ± 0.0	100 ± 0.0	100 ± 0.0	47 ± 2.1
Salmonella paratyphi	26 ± 1.1	38 ± 1.6	52 ± 2.3	67 ± 3.1	79 ± 3.6	92 ± 4.2	100 ± 0.0	53 ± 1.8
Vibrio cholera	47 ± 2.2	74 ± 3.4	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Vibrio vulnificus	32 ± 1.5	49 ± 2.3	67 ± 3.2	84 ± 4.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Staphylococcus aureus	15 ± 0.6	22 ± 0.9	29 ± 1.3	36 ± 1.6	43 ± 2.1	50 ± 2.4	56 ± 2.6	100 ± 0.0
Shigella boydii	5 ± 0.2	8 ± 0.3	11 ± 0.4	14 ± 0.6	16 ± 0.7	19 ± 0.9	21 ± 0.9	49 ± 2.1
Shigella dysenteriae	7 ± 0.2	11 ± 0.4	14 ± 0.6	17 ± 0.8	20 ± 0.9	24 ± 1.1	27 ± 1.2	100 ± 0.0
Pseudomonas aeruginosa	19 ± 0.8	28 ± 1.2	37 ± 1.5	46 ± 2.1	55 ± 2.6	64 ± 2.9	73 ± 3.4	100 ± 0.0
Escherichia coli	29 ± 1.3	44 ± 1.9	58 ± 2.7	73 ± 3.3	87 ± 4.1	100 ± 0.0	100 ± 0.0	100 ± 0.0
Bacillus cereus	35 ± 1.6	53 ± 2.4	69 ± 3.3	86 ± 4.1	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Proteus mirabilis	41 ± 1.8	59 ± 2.6	82 ± 3.7	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	51 ± 2.2
Klebsiella oxytoca	9 ± 0.4	14 ± 0.7	19 ± 0.9	24 ± 1.1	29 ± 1.3	33 ± 1.5	37 ± 1.7	56 ± 2.3

Staphylococcus aureus, *Klebsiella oxytoca*, *Shigella dysenteriae*, and *Shigella boydii*, and the highest growth inhibition of 73 ± 3.4%, 56 ± 2.6%, 37 ± 1.7%, 27 ± 1.2%, and 21 ± 0.9%, respectively which was obtained at the highest concentrations tested in this study (200 µg/ml). At the same concentration (200 µg/ml), the standard reference antibiotic standard, Linezolid showed complete growth inhibition against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *and Shigella dysenteriae*, respectively. This observation showed unique antimicrobial activities of the lipoxazolidinone A when compared to the same class of oxazolidinone antibiotic, Linezolid, which revealed the significance of this study. Microscopic observation was carried out using CLSM to examine the antimicrobial activities of lipoxazolidinone A. Staining was performed with a fluorescent dye, acridine orange, after the antimicrobial treatment at 48 hrs, and the microscopic observations of all the important concentrations are represented in Figs 1 and 2.

During the observation, growth controls showed dense bacterial biomass, and the concentrations recorded complete inhibition as evidenced in the antimicrobial assay, revealing no bacterial cells. Microscopic observations revealed that cell density was inversely proportional to the concentration of lipoxazolidinone A. Specifically, as the concentration of lipoxazolidinone A increased, the bacterial cell density decreased. This finding strongly correlates with the values obtained from the antimicrobial assays conducted in this study. Further, this microscopic observation serves as a secondary confirmation of the antimicrobial potential of lipoxazolidinone A. Similarly, in an earlier study, Lipoxazolidinone A purified from a marine actinobacterium, NPS008920, revealed antimicrobial activity against multidrug-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium.*[22] In another study, Lipoxazolidinone A was extensively identified for its antimicrobial activity against gram-negative pathogens.[20] In support of the present study, a novel compound dimannooleate isolated from a marine *Staphylococcus saprophyticus* SBPS-15 revealed promising antimicrobial activity against various clinically isolated human pathogens.[30] Moreover, the antimicrobial activity of Lipoxazolidinone A was first examined in this study against the following pathogens, viz., *Salmonella typhi*, *Salmonella paratyphi*, *Shigella boydii*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Proteus mirabilis*, and *Klebsiella oxytoca*, respectively. The findings from these studies suggest that the bioactive compound Lipoxazolidinone A has promising potential as an antibiotic.

Cytotoxicity of Lipoxazolidinone A against Normal Mammalian Cell Line Cultures

Toxicity evaluations of bioactive compounds, including antimicrobials, are crucial for understanding the adverse side effects of drugs intended for healthcare purposes.[31,32] In light of this, it is an essential and preliminary step to do cytotoxicity studies against newly emerging drugs, especially using mammalian cells, as this represents an inevitable step toward preclinical testing.[15,33,34] In this study, the cytotoxicity of lipoxazolidinone A was tested against two different normal mammalian cell line cultures; they were primary mouse embryonic fibroblast cells (NIH/3T3) and Madin-Darby Canine kidney cells (MDCK). Various concentrations of lipoxazolidinone A were tested within 50–400 µg/ml range along with positive and negative controls (Table 2). These concentrations were selected for the cytotoxicity assay based on the findings from the antimicrobial assay, viz., the highest concentration achieved complete growth inhibition against most of the pathogenic strains (200 µg/ml), the double strength of the highest tested concentration (400 µg/ml) and several intermediate concentrations were also examined for confirmation.

No tested concentrations in this study showed cytotoxicity against the two normal mammalian cells, even the highest tested concentration of 400 µg/ml revealed no cytotoxicity. Furthermore, the microscopic observations using the two fluorescent stains also confirmed the same results in which the dead cells or red colour cells were observed only in the negative controls, and green colour cells were observed positive as well as in the test samples of lipoxazolidinone A (Fig 3). This proves the nontoxic activities and the biocompatibility of lipoxazolidinone A towards mammalian cells with appreciable selective inhibitory activities against infant diarrheal pathogenic strains. Moreover, this is the first report on the toxicity evaluations of lipoxazolidinone A against mammalian cells.

Similar to this study, an antimicrobial compound, trigalactomargarate purified from a marine yeast, *Cyberlindnera saturnus* strain SBPN-27, showed excellent antimicrobial activities against many

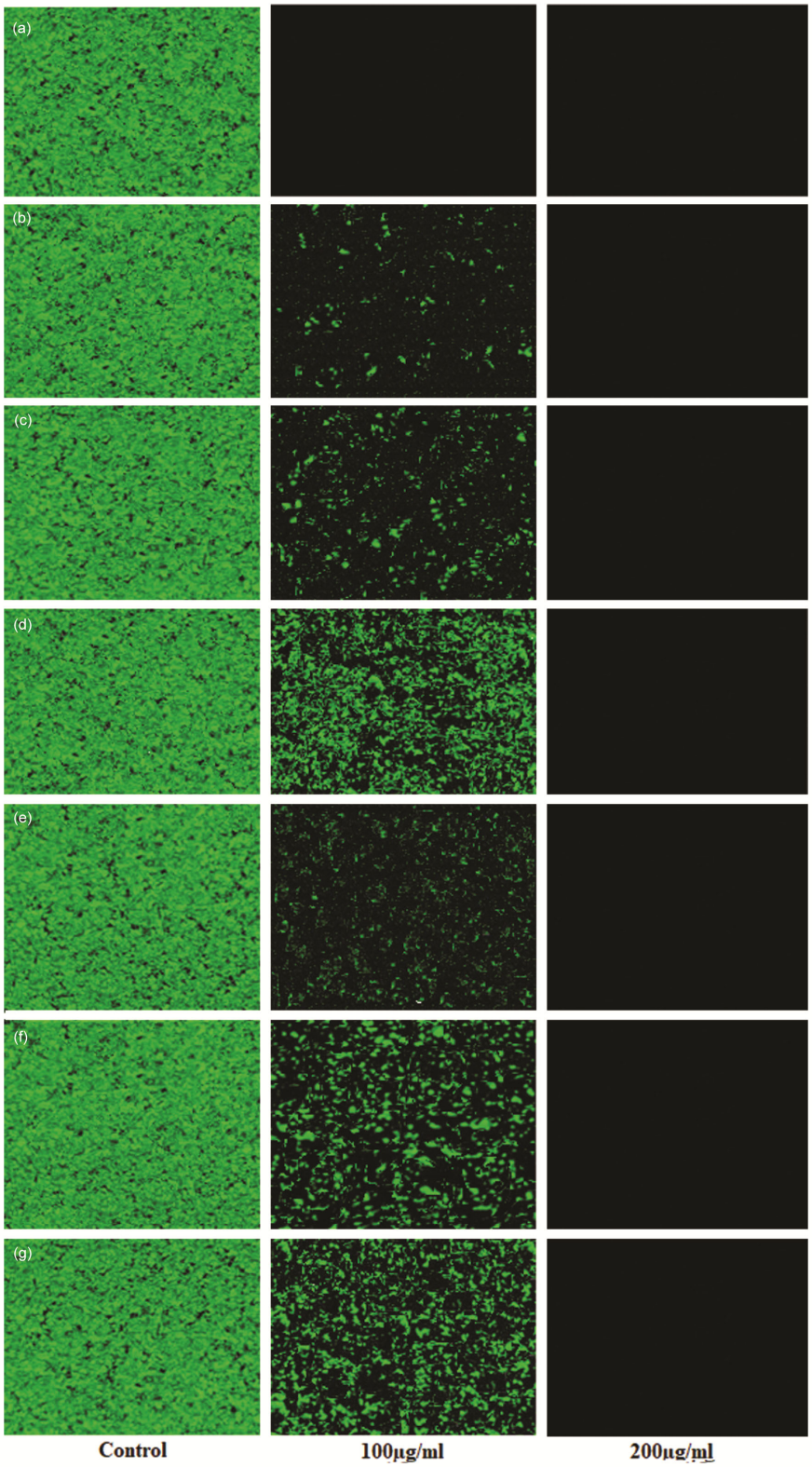

Fig. 1 — CLSM images of pathogens showing complete and partial growth inhibition at the highest (200 µg/ml) and intermediate (100 µg/ml) concentrations of Lipoxazolidinone A against: (a) *Vibrio cholerae,* (b) *Proteus mirabilis*, (c) *Salmonella typhi,* (d) *Vibrio vulnificus,* (e) *Bacillus cereus,* (f) *Escherichia coli,* and (g) *Salmonella paratyphi*

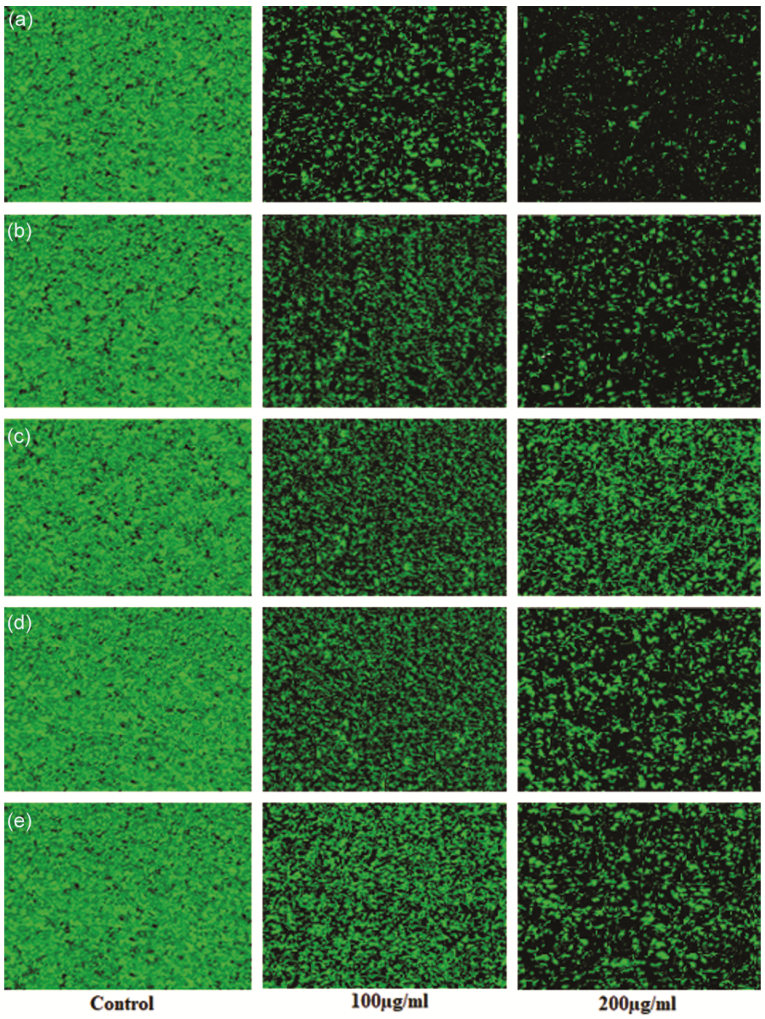

Fig. 2 — CLSM images of pathogens showing partial growth inhibition at the highest (200 µg/ml) and intermediate (100 µg/ml) concentrations of Lipoxazolidinone A against: (a) *Pseudomonas aeruginosa*, (b) *Staphylococcus aureus*, (c) *Klebsiella oxytoca*, (d) *Shigella dysenteriae*, and (e) *Shigella boydii*

Table 2 — Cytotoxicity of the Lipoxazolidinone A against the NIH/3T3 and MDCK cell lines evaluated using MTT assay

	S. No.	Lipoxazolidinone A concentration	Cell growth (%)	Cell toxicity (%)
IH/3T3 cell line		50 µg/ml	100 ± 0	0 ± 0
		100 µg/ml	100 ± 0	0 ± 0
		200 µg/ml	100 ± 0	0 ± 0
		300 µg/ml	100 ± 0	0 ± 0
		400 µg/ml	100 ± 0	0 ± 0
		Positive control	100 ± 0	0 ± 0
		Negative control (5% SLS)	0.9 ± 0.1	99.1 ± 0.1
MDCK cell line		50 µg/ml	100 ± 0	0 ± 0
		100 µg/ml	100 ± 0	0 ± 0
		200 µg/ml	100 ± 0	0 ± 0
		300 µg/ml	100 ± 0	0 ± 0
		400 µg/ml	100 ± 0	0 ± 0
		Positive control	100 ± 0	0 ± 0
		Negative control (5% SLS)	2.9 ± 0.2	97.1 ± 0.4

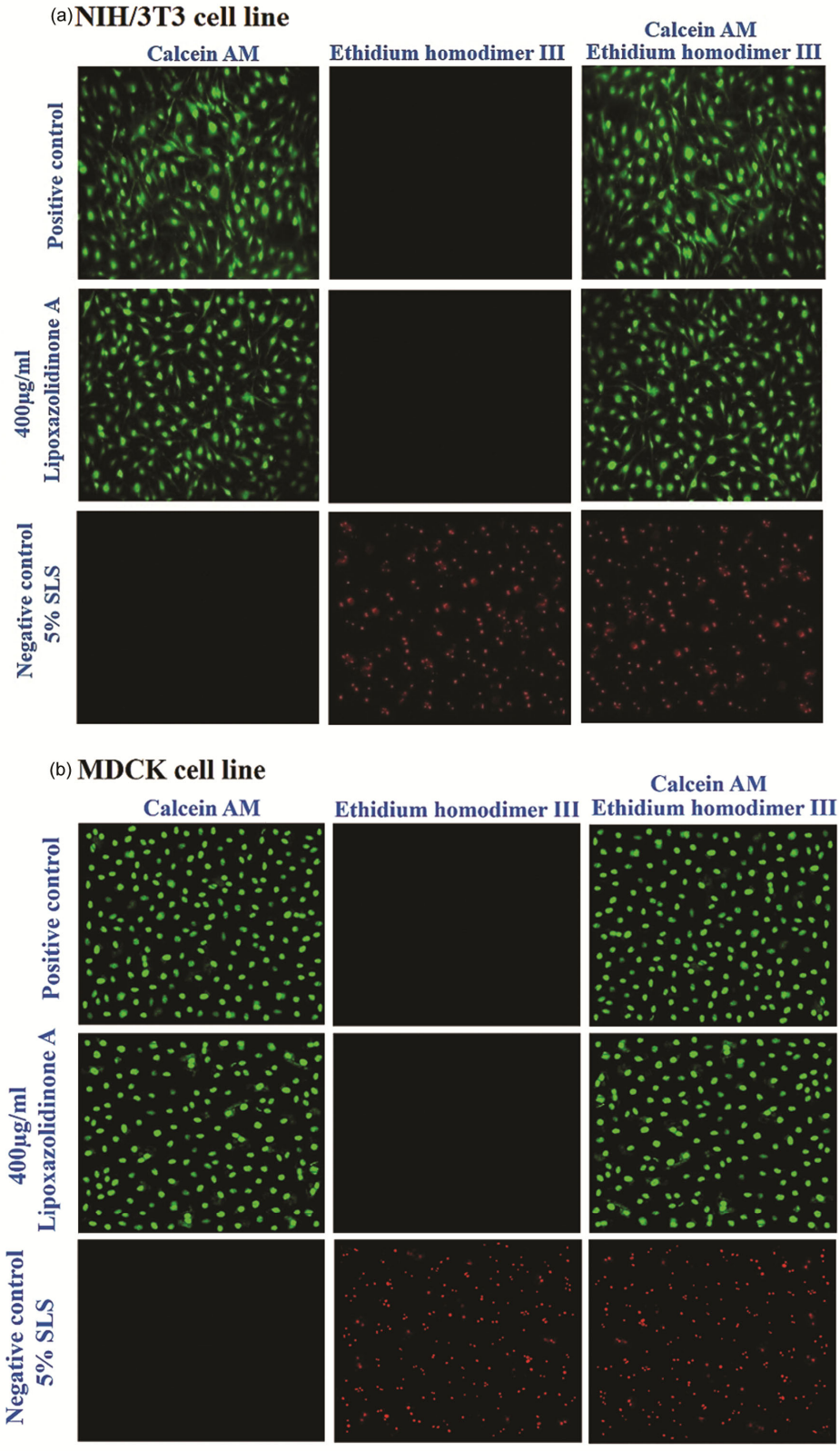

Fig. 3 — Cytotoxicity of the Lipoxazolidinone A against: (a) NIH/3T3 and (b) MDCK cell line culture under fluorescence microscopy using Calcein AM (fluorescent green colour denotes viable cells) and Ethidium homodimer III (fluorescent red colour denotes dead cells)

clinically isolated human pathogens with negligible toxicity against mammalian 3T3 fibroblast cells.[35] Likewise, protein-derived peptide Cp1 synthesised from bovine α_{S1}-casein revealed appreciable antimicrobial activity against both gram-positive and negative strains and, at the same time, showed no toxicity against mammalian cell line culture, human embryonic kidney (HEK293) cells.[36] In another study, ethylene glycolylated poly (amidoamine) dendrimers revealed appreciable antimicrobial activities against common ocular pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and showed negligible toxicity against human corneal epithelial cells.[37]

Conclusions

This study explores the antimicrobial activities and cytotoxicity assessment of lipoxazolidinone A purified from a probiotic strain, *Lactobacillus apis* YMP3. This bioactive compound showed appreciable growth inhibition activities against various infant diarrheal infectious pathogens and revealed negligible cytotoxicity towards mammalian cells. These findings suggest that Lipoxazolidinone A is safe for mammalian cells at effective antimicrobial concentrations, positioning it as a promising candidate for drug development. These observations expand the identification of promising bioactive compounds from Lactobacillus species and valuable microbes found in traditional fermented foods. Additionally, the identifications carried out in this study served as baseline data for more extensive research on the therapeutic potential of Lipoxazolidinone A in treating various gastrointestinal infections in infants.

Conflict of interest

The authors declare that they have no conflict of interest in the publication of this article.

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