ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 9, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

THE UNEXPLORED ROLES OF PROBIOTIC BACTERIA: *IN VITRO* ANTI-INFLAMMATORY AND ANTHELMINTIC ACTIVITY OF *ENTEROCOCCUS FAECIUM* BM10 KY788342 AND *LACTOBACILLUS CASEI* GM10 KY794586

NEHA JAIN*, ARCHANA MEHTA

Department of Botany, Laboratory of Molecular Biology, Dr. H. S. Gour Central University, Sagar - 470 003, Madhya Pradesh, India.
Email: nehaj147@gmail.com

Received: 26 April 2017, Revised* and *Accepted: 20 May 2017

ABSTRACT

Objective: The aim of the present study was to evaluate the *in vitro* anti-inflammation activity and anthelmintic potential of two novel isolated probiotic strains through *Enterococcus faecium* BM10 KY788342 and *Lactobacillus casei* GM10 KY794586.

Methods: *In vitro* anti-inflammatory activity was evaluated using protein denaturation inhibition method. *Pheretima posthuma* was used as a suitable *in vitro* model, and time of paralysis and death were used as parameters to evaluate anthelmintic potential of probiotic strains.

Results: Lyophilized solutions of *L. casei* GM10 showed significant protein denaturation inhibition (56.20±0.86%) followed by *E. faecium* BM10 (52.28±0.31%) comparable to diclofenac (93.62±1.39%) at the maximum concentration of 250 µg/ml. Intracellular cell-free extract of *E. faecium* BM10 showed a strong anthelmintic (vermicidal) activity (6±0.23 minutes), followed by *L. casei* GM10 (9±0.05 minutes) comparable to piperazine citrate (20±0.422 minutes) and albendazole (24±0.43 minutes) at the maximum concentration of 100 mg/ml.

Conclusion: Results of the present study concluded that both tested lactic acid bacteria strains exhibited significant *in vitro* anti-inflammatory activity and can be used as potent and safe anthelmintic agent.

Keywords: Lactic acid bacteria, Anti-inflammatory, Anthelmintic, Intracellular cell-free extract.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i9.19413

INTRODUCTION

Intestinal inflammation and infections are generally accompanied due to an imbalance of intestinal microflora. Probiotic bacteria play an important role in balancing the gut natural microflora and now used as the alternative approach of prevention and therapy for numerous intestinal inflammatory disorders, including inflammatory bowel disease. The mechanisms associated with probiotics include blocking of pathogenic bacterial effects by producing antibacterial compounds; competitive inhibition of pathogens and toxins from adhesion to intestinal epithelium; regulate mucosal immune response through improving intestinal immunological barrier (mainly IgA response), enhancing host innate immunity; and maintain a control between pro- and anti-inflammatory cytokines [1,2]. Furthermore, *Lactobacillus rhamnosus* GG MTCC 1408 and lyophilized cell-free supernatant of *Enterococcus faecium* CFR 3003 exhibited a strong anti-inflammatory effects by downregulating the tumor necrosis factor (TNF) - a production and increasing the IL-10 levels in lipopolysaccharide-stimulated macrophage cell lines [3].

Helminthic infections are generally caused by soil-transmitted helminths. These infections are common in those areas where inadequate sanitation and unsafe water supplies are prevalent. These parasitic worms also infect livestock and crops. According to latest estimates, more than 2 billion people are infected with these parasites [4]. Although many synthetic drugs including preparation, benzimidazole, imidazothiazole, and albendazole are available in the market, and in addition to it, various herbal plant parts also utilized as an anthelmintic agent, but these synthetic drugs are losing their effectiveness as resistance develops in nematodes against drugs [5]. Only a few studies have been conducted regarding the use of bacterial metabolites as anthelmintic [6-8], therefore in the present study, two probiotic strains: *E. faecium* BM10 KY788342 and *Lactobacillus casei* GM10 KY794586 evaluated for anthelmintic activity and anti-inflammatory activity.

METHODS

In vitro anti-inflammatory activity

In vitro anti-inflammatory activity was performed using protein denaturation inhibition assay [9]. Reaction mixture (0.5 ml) contains 0.45 ml of bovine serum albumin (BSA) (5% w/v aqueous solution) and 0.05 ml of lyophilized lactic acid bacteria (LAB) test sample (50, 100, and 250 µg/ml) in citrate buffer. Test control solution (0.5 ml) comprised 0.45 ml of BSA (5% w/v aqueous solution) and 0.05 ml of distilled water, whereas product control solution (0.5 ml) consisted of 0.45 ml of distilled water and 0.05 ml of test solution (50, 100, and 250 µg/ml). Standard solution (0.5 ml) prepared by 0.45 ml of BSA (5% w/v aqueous solution) and 0.05 ml of diclofenac sodium (50, 100, and 250 µg/ml). pH of all solutions adjusted to 6.5. The samples were incubated at 37°C for 20 minutes, and then the temperature was increased to 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer saline was added to the above solutions. The absorbance was measured at 416 nm. The percentage inhibition of protein denaturation was calculated as

$$\text{Protein denaturation inhibition (\%)} = (OD_t/OD_c\text{-}1) \times 100$$

Anthelmintic activity

Preparation of intracellular cell-free extract (ICFE)

ICFE of both LAB was prepared according to the study by Chen *et al*. [10]. 10 µl of LAB culture was inoculated in 250 ml of De Man-Rogosa Sharpe broth medium and incubated for 3 days at 30ºC. The bacterial counts in the cell suspension (intact cells) were adjusted to 10^8 cfu/ml. The cell suspensions were subjected to ultrasonic disruption (five 1-min intervals in an ice bath), the cell debris was removed by centrifugation

(10,000 ×g) for 10 minutes at 4°C, and the prepared resulting supernatant (ICFE) was collected and used for the study.

Procedure

Anthelmintic activity was carried out according to the method suggested by Bharti *et al*. [11]. Indian adult earthworms (*Pheretima posthuma*) were used for the study as these have anatomical and physiological resembles with intestinal roundworm parasites of human being. Earthworms were collected from moist soil and washed with normal saline and used for the study. The ICFE of both bacteria were dissolved in 20 mm citrate phosphate buffer and prepared desired concentrations (25, 50, and 100 mg/ml). Piperazine citrate and albendazole were used as the standard drugs. 13 groups containing 6 earthworms in each group were placed in petri dishes containing 15 ml test and standard formulation. Group 1 served as control (0.9% NaCl), Groups 2, 3, and 4 treated with piperazine citrate (25, 50, and 100 mg/ml), Groups 5, 6, and 7 treated with albendazole (25, 50, and 100 mg/ml), Groups 8, 9, and 10 treated with ICFE of *E. faecium* GM10 (25, 50, and 100 mg/ml), and Groups 11, 12, and 13 treated with ICFE of *L. casei* GM10 (25, 50, and 100 mg/ml). Observations were made for the time taken for paralysis and death of individual worm. The paralysis was assumed to occur when the worms were not able to move even in normal saline while the death was concluded when the worms lost their motility followed with fading away from their body colors. Assay was conducted in triplicates.

RESULTS

Anti-inflammatory activity

In the present study, *in vitro* anti-inflammatory activity of a lyophilized solution of *E. faecium* BM10 and *L. casei* GM10 was determined by percentage inhibition of protein denaturation method and compared with standard drug Diclofenac. *E. faecium* BM10 showed protein denaturation inhibition (%) 45.10±0.16, 49.58±0.50, and 52.28±0.31 at the concentration of 50, 100, and 250 µg/ml, respectively. However, *L. casei* GM10 exhibited protein denaturation inhibition (%) 48.15±1.78, 51.34±1.80, and 56.20±0.86 at the concentration of 50, 100, and 250 µg/ml, respectively. The standard drug Diclofenac showed protein denaturation inhibition (%) 88.02±0.98, 90.11±1.06, and 93.62±1.39 at the concentration of 50, 100, and 250 µg/ml (Fig. 1). Both LAB strains showed concentration-dependent inhibition of protein denaturation, and *L. casei* GM10 considered being a stronger anti-inflammatory agent than *E. faecium* BM10.

Anthelmintic activity

In the present investigation, ICFEs of *E. faecium* BM10 and *L. casei* GM10 were tested for anthelmintic activity using *Pheretima posthuma* model, and the results were compared with standard drugs: Piperazine citrate and albendazole. The LAB strains and standard drugs showed concentration-dependent anthelmintic activity. The paralysis (vermifuge) and death time (vermicidal) of ICFE of *E. faecium* BM10 were 6±0.029 minutes, 4±0.017 minutes, and 3±0.10 minutes and 10±0.16 minutes, 9±0.32 minutes, and 6±0.23 minutes at the concentration of 25, 50, and 100 mg/ml, respectively. However, the paralysis and death time of ICFE of *L. casei* GM10 were 11±0.71 minutes, 9±0.34 minutes, and 6±0.03 minutes and 18±0.48 minutes, 15±0.22 minutes, and 9±0.05 minutes at the concentration of 25, 50, and 100 mg/ml, respectively. The paralysis and death time of worms showed by standard drug piperazine citrate were 30±0.87 minutes, 21±0.61 minutes, and 14±0.30 minutes and 42±1.03 minutes, 35±0.35 minutes, and 20±0.422 minutes at the concentration of 25, 50, and 100 mg/ml, respectively. The paralysis and death time of worms showed by albendazole were 35±0.76 minutes, 27±0.23 minutes, and 18±0.92 minutes and 50±1.57 minutes, 40±0.88 minutes, and 24±0.43 minutes at the concentration of 25, 50, and 100 mg/ml, respectively. The maximum anthelmintic activity was showed by ICFE of *E. faecium* BM10 and minimum by albendazole (Fig. 2).

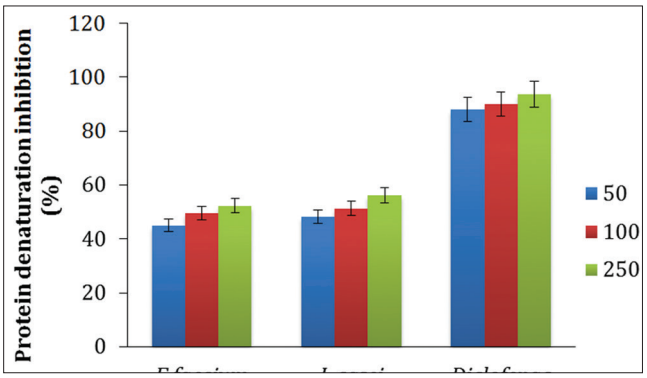

Fig. 1: Anti-inflammatory activity (%) of *Enterococcus faecium* BM10 and *Lactobacillus casei* GM10 compared to diclofenac (standard drug)

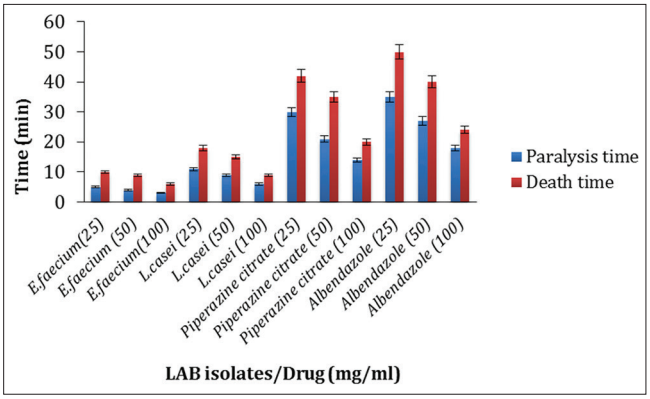

Fig. 2: Anthelmintic activity of *Enterococcus faecium* BM10 and *Lactobacillus casei* GM10 compared to standard drugs (piperazine citrate and albendazole)

DISCUSSION

Denaturation of tissue proteins is a well-documented cause of inflammation [12] and arthritic disease. The inflammatory drugs (salicylic acid, phenylbutazone, etc.,) have shown dose-dependent ability to thermally induced protein denaturation [13]. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat-treated albumin at the physiological pH (pH: 6.2-6.5). Results of anti-inflammatory activity revealed that *E. faecium* BM10 and *L. casei* GM10 decrease protein denaturation significantly. Recently, Williams *et al*. [14] demonstrated that *L. reuteri* BM36301 suppressed TNF-α induction and decreased inflammation in aged mice. Similarly, Carroll *et al*. [15] investigated the anti-inflammatory role of $MnSO_4$ released from *Lactobacillus gasseri* in interleukin 10-deficient colitis mouse. Mostly previously reported anti-inflammatory activities were assessing medicinal herbs as anti-inflammatory agents [16,17]. Our study first time reported the *in vitro* anti-inflammatory activity of probiotic strains and exhibited significant results of anti-inflammatory activity.

'Avermectin' a series of compounds contains high degree of anthelmintic and anti-parasitic activity, isolated from *Streptomyces avermitilis*, and many metabolites have been isolated from bacteria (*Brevibacillus laterosporus* strain G4 and *Bacillus sp*. B16) and fungi *(Arthrobotrys oligospora, Cylindrocarpon destructans, Verticillium chlamydosporium*, and *Paecilomyces lilacinus*) also showed antinematode activity [18-20]. Our study showed strong anthelmintic activity of ICFE of both tested LAB strains (*E. faecium* BM10 and *L. casei* GM10) and considered more effective than piperazine citrate and albendazole. Similar results also

reported by Bharti *et al.,* [11] *L. plantarum* and *L. acidophilus* exhibited 100% paralysis of worms within 48 hrs. Extract containing metabolites of *Bacillus cereus* and *Bacillus pumilus* also showed significant anthelmintic activity in paralyzing the worms. Probiotic bacteria have a symbiotic relation in the human gut and tested LAB strains showed good acid and bile salt tolerance activity in the previous reported study, therefore our study to assess the anthelmintic activity based on the fact that the LAB strain can kill intestinal worms without affecting natural gut balance and would not show any resistance in anthelmintic due to having human origin.

CONCLUSION

In vitro anti-inflammatory activity and anthelmintic activity of tested strains are the novel studies employed for assessing therapeutic applications of *E. faecium* BM10 and *L. casei* GM10 strains. Lyophilized LAB solution of tested strains showed significant anti-inflammatory activity as well as ICFE of both tested probiotic strains exhibited potent anthelmintic activity. *In vitro* studies are the first step to assess any microbe or compound for their therapeutic potential followed by *in vivo* activities. Significant findings of *in vitro* assay assured and forced for conducting *in vivo* activities, and future studies of tested LAB strains will be carried out in this direction.

ACKNOWLEDGMENT

The authors would like to express their grateful to the Department of Botany, Dr. H. S. Gour (Central) University, Sagar (M.P.), for providing necessary facilities to carry out this work and also thanking full to the DST INSPIRE, for providing a grant for this work.

REFERENCES

1. Ménard S, Candalh C, Bambou JC, Terpend K, Cerf-Bensussan N, Heyman M. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. Gut 2004;53(6):821-8.
2. Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P. Anti-inflammatory activity of *Lactobacillus* on carrageenan-induced paw edema in male wistar rats. Int J Inflam 2012;2012:752015.
3. Divyashri G, Krishna G, Muralidhara, Prapulla SG. Probiotic attributes, antioxidant, anti-inflammatory and neuromodulatory effects of *Enterococcus faecium* CFR 3003: *In vitro* and *in vivo* evidence. J Med Microbiol 2015;64(12):1527-40.
4. World Health Organization. Soil-Transmitted Helminthiases: Eliminating Soil-Transmitted Helmnthiases as a Public Health Problem in Children. Progress Report; 2012. p. 1-90.
5. Holden-Dye L, Robert WJ. Anthelmintic drugs and nematicides: Studies in *Caenorhabditis elegans*. In: Worm Book: The Online Review of *C. Elegans* Biology. New York, USA: W. H Freeman; 2013. p. 1-22.
6. Kumar ML, Thippeswamy B, Kuppust IL, Naveenkumar KJ, Shivakumar CK. Evaluation of *Bacillus cereus* and *Bacillus pumilus* metabolites for anthelmintic activity. Pharmacognosy Res 2015;7(1):81-4.
7. Haber CL, Heckaman CL, Li GP, Thompson DP, Whaley HA, Wiley VH. Development of a mechanism of action-based screen for anthelmintic microbial metabolites with avermectinlike activity and isolation of milbemycin-producing *Streptomyces* strains. Antimicrob Agents Chemother 1991;35(9):1811-7.
8. Amanto JS, Brooklyn NY, Cvetovich R, Scotch, P. United States Patent No. 19; 1995.
9. Kar B, Nepal A, Kumar RB, Dolai N, Bhattacharya S, Upal K, *et al*. Antioxidant and anti-inflammatory properties hymenodictyon excelsum bark. Orient Pharm Exp Med 2013;13(2):103-11.
10. Chen Q, Kong B, Sun Q, Dong F, Liu Q. Antioxidant potential of a unique LAB culture isolated from Harbin dry sausage: *In vitro* and in a sausage model. Meat Sci 2015;110:180-8.
11. Bharti V, Mehta A, Jain N, Singh S, Ahirwal L. *In vivo* acute toxicity, antibacterial, antiaquatic fungal, antihelminthic activity of *Lactobacillus plantarum* kp894100 and *Lactobacillus acidophilus*. Asian J Pharm Clin Res 2016;9(1):1-5.
12. Opie EL. On the relation of necrosis and inflammation to denaturation of proteins. J Exp Med 1962;115(3):597-608.
13. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol 1968;20(3):169-73.
14. Williams LA, Connar AO, Latore L, Dennis O, Ringer S, Whittaker JA, *et al*. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of anim. West Indian Med J 2008;57(4):327-31.
15. Carroll IM, Andrus JM, Bruno-Bárcena JM, Klaenhammer TR, Hassan HM, Threadgill DS. Anti-inflammatory properties of *Lactobacillus gasseri* expressing manganese superoxide dismutase using the interleukin 10-deficient mouse model of colitis. Am J Physiol Gastrointest Liver Physiol 2007;293(4):G729-38.
16. Saraswathi R, Upadhyay L, Venkatakrishnan R, Meera R, Devi P. Phytochemical investigation, analgesic and anti-inflammatory activity of *Abutilon indicum* Linn. Int J Pharm Pharm Sci 2011;3:154-6.
17. Nagore DH, Ghose VK, Patil MJ, Wahile AM. *In vitro* antioxidant and *in vivo* anti-inflammatory activity of *Cassia sophera* Linn. Int J Pharm Pharm Sci 2010;2:113-21.
18. Giblin-Davis RM, Williams DS, Bekal S, Dickson DW, Brito JA, Becker JO, *et al.* 'Candidatus pasteuria usgae' sp. Nov. An obligate endoparasite of the phytoparasitic nematode *Belonolaimus longicaudatus*. Int J Syst Evol Microbiol 2003;53:197-200.
19. Dash GK, Suresh P, Sahu SK, Kar DM, Ganapaty S, Panda SB. Evaluation of *Evolvulus alsinoides* Linn. For anthelmintic and antimicrobial activities. J Nat Rem 2002;2(2):182-5.
20. Partap S, Kumar S, Kumar A, Sharma NK, Jha KK. *In-vitro* anthelmintic activity of *Luffa cylindrica* leaves in Indian adult earthworm. J Pharmacogn Phytochem 2012;1(2):27-30.