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Screening of lactobacilli cultures for their antibacterial activity against food borne pathogens

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Abstract
Different lactobacilli isolates were studied for antibacterial activity against food pathogens by employing agar well assay method. All broth cultures of eighteen isolates of lactobacilli and yoghurt cultures were screened for antibacterial activity by agar diffusion technique showed positive effect against *E. coli*. Only seven isolates of lactobacilli and yoghurt cultures were screened for antibacterial activity against *Salmonella* Spp and *Shigella dysentriae*, *Lb. acidophilus* exhibited maximum activity followed by yoghurt culture. Among the three test pathogens used, *E. coli* found to be more susceptible followed by *Salmonella* spp. and *Shigella dysentriae* for the antibacterial substance produced by *Lb. acidophilus* and yoghurt culture.

Keywords: Screening, lactobacilli, cultures, antibacterial, pathogens

1. Introduction
Lactobacilli are the members of Lactic acid bacteria (LAB), are gram-positive, non-spore forming rods with an ability to produce lactic acid as a main end product of their carbohydrate metabolism. About eighty species lactobacilli are recognized at present. They are catalase-negative, aerotolerant or anaerobic in nature. These bacteria produce antimicrobial molecules like ethanol, bacteriocins, hydrogen peroxide, fatty acid to exert an antibacterial activity. By these mechanisms they inhibit several bacterial pathogens including *Clostridium difficile*, *Escherichia coli*, *Staphylococcus aureus*, and *Shigella* spp. The antibacterial activity is determined by using different techniques like agar well assay, disc method, tube dilution method and microtitre wells methods. In this study cell free supernatant of broth cultures of lactobacilli is used in agar well assay method to assess the antibacterial activity of the cultures.

2. Materials and Methods
2.1 Lactic cultures
Lactic acid bacteria were used in this study are *Lactobacillus acidophilus* (C1, C2, C3,C4, D1, D2), *Lactobacillus brevis* (C5), *Lactobacillus fermentum* (C6, C7, C8, C9), *Lactobacillus delbrueckii spp. delbrueckii*, (G1) *Lactobacillus delbrueckii* (D3, D4, U1), *Lactobacillus delbrueckii spp. lactis* (G2), *Lactobacillus delbrueckii spp.bulgaricus* (C10), *Lactobacillus animalis* (D5), *Lactobacillus lactis ssp. lactis, Lactobacillus lactis ssp. cremoris, Lactobacillus lactis ssp. lacts var. diacetylactis, Leuconostoc ssp. Streptococcus salivarius ssp. thermophilus.* All the above lactic cultures were obtained from culture collection of postgraduate laboratory, Dept. of Dairy Microbiology, KVAFSU Bangalore.

2.2 Indicator organisms for testing antibacterial activity
The following enteropathogens, maintained at postgraduate laboratory, Dept. of Dairy Microbiology, KVAFSU Bangalore, were used for antibacterial assay. *E. coli, Shigella dysentriae,* and *Salmonella spp.*

2.3 Yeast glucose agar as antibacterial assay medium
Yeast glucose agar (YGA) was prepared by dissolving in 15 g of agar in 1000 ml of yeast glucose broth and sterilized at 121 ^{0}C for 15 min. D-Glucose content was reduced from 0.5% to 0.25% to overcome the problem of gas pockets in antibacterial assay plates.

2.4 Maintenance of cultures
2.4.1 Broth cultures
Stock cultures of lactobacilli were maintained in deMan Rogosa Sharpe (MRS) agar stabs.

Yoghurt culture, lactococci and leuconostocs were maintained in YGA stabs. These cultures were subcultured once in a month. Between subculturing, the cultures were stored in a deep freezer. Working cultures of lactobacilli were maintained in MRS broth. Yoghurt culture, lactococci and leuconostocs were maintained in YG broth. These cultures were subcultured once in a week. Between subculturing, the cultures were stored in refrigerator.

2.4.2 Food pathogens

Stock culture of *E. coli, Shigella dysentriae, Salmonella spp.* were maintained on YGA slant and subcultured once in a month. Between subculturing the culture were stored in a deep freezer. Working cultures were stored in YG broth, subcultured once in week and stored in a refrigerator.

2.5 Preparation of culture filtrate

MRS broth (10 ml) was inoculated with 0.1 ml of active culture of lactobacilli. The tubes were incubated at 37 ^{0}C anaerobically for 72 h, after the completion of incubation period cultures were centrifuged aseptically at 10,000 rpm for 15 min. The supernatant was filter sterilized and used for detection of antibacterial activity.

2.6 Preparation of agar plates

Food pathogens were grown in YG broth at 37 ^{0}C for 18 hr. One ml of this broth culture was added to 9 ml of sterilized saline solution and mixed well. Total number of cells present in this diluted sample was counted by following the direct microscopic count technique (IS: SP: 18, Part XI, 1981). Based on DMC of activity broth culture, the quantity of broth culture to be taken was fixed. Appropriate volume of pathogen culture, was then seeded into YG agar (melted and cooled at 50 ^{0}C) to get a final count of 10^5 cells per ml of agar. This seeded agar (20 ml) was then poured into a 9 cm diameter antibiotic assay petri plate. Agar was allowed to set, made to become firm by keeping at 4 ^{0}C for 1 hr and then condensate formed on upper part of petri plate was removed by flaming on burner aseptically. These plates were further for assay technique.

2.7 Screening of lactobacilli for antibacterial activity

All the *lactobacilli* cultures were screened for their antibacterial activity by agar diffusion technique, against enteropathogens *E. coli, Shigella dysentriae* and *Salmonella spp*.

The technique as standardized by Prabha (1984) [5], was followed for the detection antibacterial activity in culture filtrates. Four wells of 7.0 mm diameter were made on each agar petri plate using a glass borer. Into these wells, 50 µl of culture filtrate was added. The plates were then incubated at 37 ^{0}C for 48 hr. without inversion. Formation of clear zone around the wells was taken as the zone of inhibition. The diameter of the inhibitory zone including the diameter of the well was measured. Area of inhibitory zone was calculated as follows and it was expressed in term of mm^2. Area of inhibitory zone = (Area of Clear Zone) - (Area of Agar Well).

3. Results and Discussion

3.1 Screening for antibacterial activity of broth cultures of lactobacilli against pathogens

In order to determine the ability of lactobacilli to produce inhibition compounds against pathogens such as *E. coli, Salmonella spp.* and *Shi. dysentriae*, lactobacilli cultures were grown in broth and cell free supernatant were screened for antibacterial activity using *E. coli* as test organism. The test pathogen was seeded (10^5 cells/ml) into YG agar and after solidification of agar, wells, 50 µl of filter-sterilized culture filtrates (which were grown in YG broth and centrifuged at 10,000 rpm for 15 min) was added and incubated. The inhibition zone was determined by considering the clear zone around the well.

The results of these studies are shown in the table 1 and fig. 1

It may be seen that all the isolates showed antibacterial activity against *E coli.* It is found that *Lb. acidophilus* C1 showed highest activity (94.20 Sq. mm) followed by the mixed culture (yoghurt culture), (85.58 Sq. mm). Among *Lb. acidophilus* strains, D2 strain showed the least activity (40.04 Sq. mm). Among the *Lb. fermentum* strains, three of them (C7, C8, C6) showed the same activity (56.52 Sq.mm) while C9 showed very low activity (11.78 Sq. mm). Among *Lb. delbrueckii isolates, Lb. bulgaricus*, C10 showed highest activity (65.35 Sq. mm) while, G1, D3, U1, G2 showed the same activity (56.52 Sq. mm) while D4 isolate showed the least activity (25.12 Sq. mm). *Lb. brevis* showed an activity of 74.58 Sq. mm. and *Lb. animalis* showed an activity of 56.52 Sq. mm. For all isolates, the pH values of culture filtrate varied from 3.0 to 3.5. Similar trend was observed with Sarkar *et al*., (1996) [7] who examined antibacterial activity of 171 LAB isolates using agar spot test and well diffusion technique and found that only 24 strains inhibited the test pathogen *E. coli*.

Among the 18 isolates of lactobacilli, one isolate from each spp. *(seven isolates)* and yoghurt culture were selected, for determining their antibacterial activity against *Salmonella spp*. and *Shi. dysentriae*.

The results against *Salmonella spp.* are shown in Table 2. and Fig. 2. It is observed that, *La. acidophilius* C1 showed the highest activity (78.58 Sq. mm) while, *Lb. animalis* D5 showed the lowest activity (32.38 Sq. mm). However, the yoghurt culture showed an activity of 65.35 Sq. mm.

It may be seen in Table 3. and Fig. 3 that *Shi. dysentriae* was inhibited by all the 7 isolates of lactobacilli. The highest inhibition was exhibited by *Lb. acidophilus* C1 (56.52 Sq. mm) and lowest being the *Lb. animalis* D5 (11.78 Sq. mm). While the yoghurt culture exhibited the activity of 48.08 Sq. mm.

It was observed that, *E.coli* was the most susceptible organism to all the culture filtrates tested, followed by *Salmonella sp.* and *Shi. dysentriae.* Similar trend was observed by Amin *et al*., (1991) [1] for the milk culture filtrate of *Lb. bulgaricus*, tested against different enteric pathogens. In general, the highest activity was exhibited by *Lb. acidophilus* C1 against all the three test organisms followed by Yoghurt culture. Results also showed that the pH of the culture filtrate remained as 3.0, even after incubation for 72 h.

Coman *et al*., (2014) [3] studied antimicrobial activity of probiotic strains *Lactobacillus rhamnosus* IMC 501. *Lactobacillus paracasei* IMC 502 and their combination 1:1 as SYNBIO was determined by using modified streak method and agar well diffusion method against six gram-positive and nine gram-negative bacterial pathogens and eight strains of yeast *Candida* Spp. Compared with the control, most of the pathogenic bacteria and yeast were inhibited by all probiotic strains tested to various degrees.

Chen *et al*., (2019) [2] studied on anti-carbapenem resistant

Enterobacteriaceae (CRE) using agar well diffusion and broth microdilution assay and found that out of 31 Lactobacilli isolates, 5 isolates displayed greatest anti-CRE activity with inhibition zone of greater than 15 mm in agar well diffusion assays. The minimal inhibitory percentages of supernatants from these five strains against CREs ranged from 10 to 30%.

Table 1: Antibacterial activity of broth cultures of lactobacilli against *E. coli.*

Name of the culture	Isolate No.	Antibacterial activity (Sq. mm) *
Lactobacillus acidophils	C1	94.20 ± 0.79
Lb. acidophils	C2	74.58 ± 0.63
Lb. acidophils	C3	74.58 ± 1.38
Lb. acidophils	C4	65.35 ± 0.55
Lb. acidophils	D1	65.35 ± 1.21
Lb. acidophils	D2	40.04 ± 0.93
Lb. delbrueckii spp. Bulgaricus with Str. Thernophilus (yogurt	YG	85.58 ± 1.78
Lb. brevis	C5	74.58 ± 1.08
Lb. delbrueckii spp. bulgaricus	C10	65.35 ± 0.55
Lb. animalis	D5	56.52 ± 1.24
Lb. delbruecueckii spp. delbrueckii	G1	56.52 ± 1.32
Lb. delbrueckii spp. lactis	G2	56.52 ± 1.51
Lb. delbrueckii	D3	56.52 ± 0.47
Lb. delbrueckii	U1	56.52 ± 1.51
Lb. delbrueckii	D4	25.12 ± 0.36
Lb. fermentum	C6	56.52 ± 1.05
Lb. fermentum	C7	56.52 ± 1.05
Lb. fermentum	C8	56.12 ± 1.28
Lb. fermentum	C9	11.78 ± 0.31

*Cultures were grown in MRS broth at 37 C / 72 h and then centrifuged and filter sterilised. Further antibacterial activity was determined using Agar well diffusion technique.

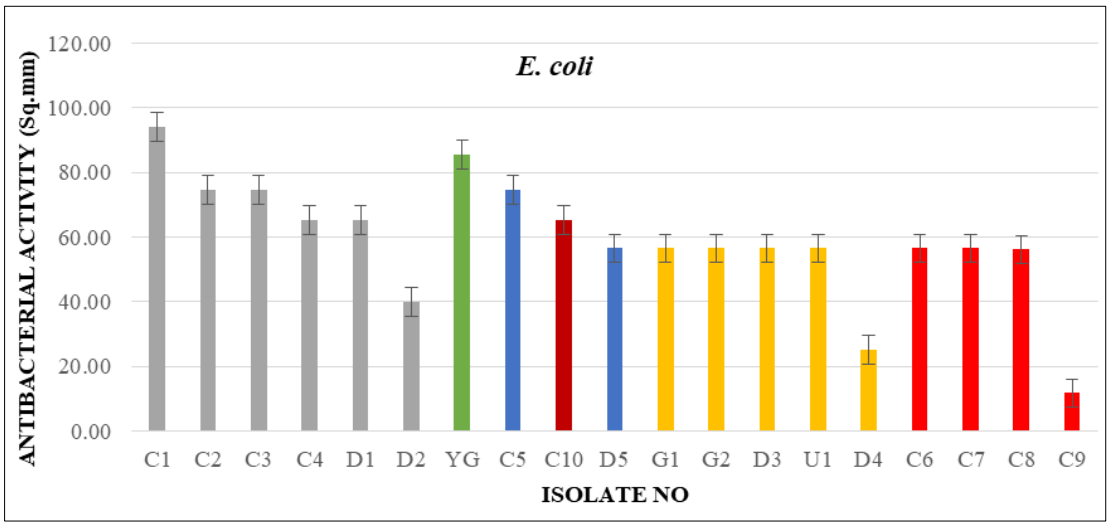

Fig 1: Antibacterial activity of lactobacillus isolates against *E. coli.*

Table 2: Antibacterial activity of broth cultures of *lactobacilli* against *Salmonella spp.*

Name of the culture	Isolate No	Antibacterial activity (sq mm)
Lactobacillus acidophilus	C1	74.58 ± 0.63
Lb. delbrueckii spp. bulgaricus with *Str. thermophilus* (yogurt culture)	YG	65.35 ± 0.55
Lb. delbrueckii spp. *bulgaricus*	C10	56.52 ± 1.05
Lb. brevis	C5	56.52 ± 0.47
Lb. delbruecueckii spp. *delbrueckii*	G1	48.08 ± 0.89
Lb. fermentum	C6	40.04 ± 0.93
Lb. delbrueckii spp. *lactis*	G2	40.04 ± 0.83
Lb. animalis	D5	32.38 ± 0.47

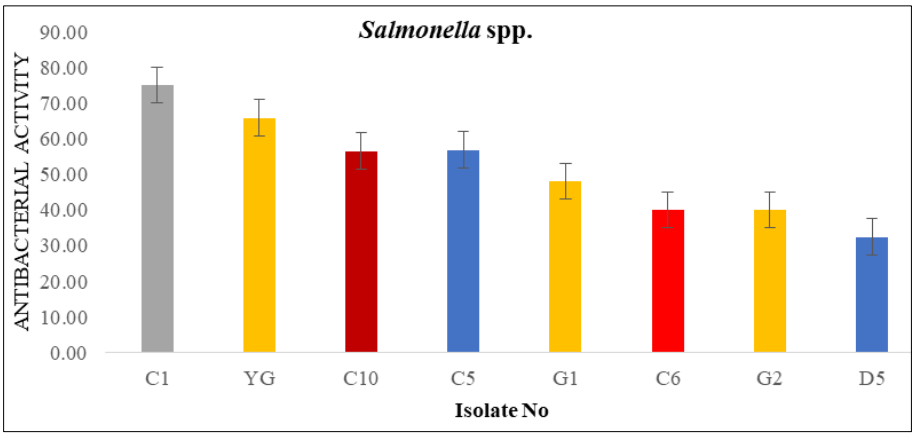

Fig 2: Antibacterial activity of *Lactobacillus* isolates against *Salmonella spp*.

Table 3: Antibacterial activity of broth cultures of lactobacilli against *Shigella dysentriae*

Name of the culture	Isolate No	Antibacterial acitivity (Sq mm)
Lactobacillus acidophils	C1	56.52 ± 0.47
Lb. delbrueckii spp. *bulgaricus* with *Str. Thermophilus* (yoghurt culures)	YG	48.08 ± 0.40
Lb. delbrueckii spp. bulgaricus	C10	40.04 ± 0.74
Lb. brevis	C5	40.04 ± 0.34
Lb. delbruecueckii spp. delbrueckii	G1	40.04 ± 0.74
Lb. fermentum	C6	25.12 ± 0.59
Lb. delbrueckii spp. lactis	G2	25.12 ± 0.52
Lb. animalis	D5	11.78 ± 0.17

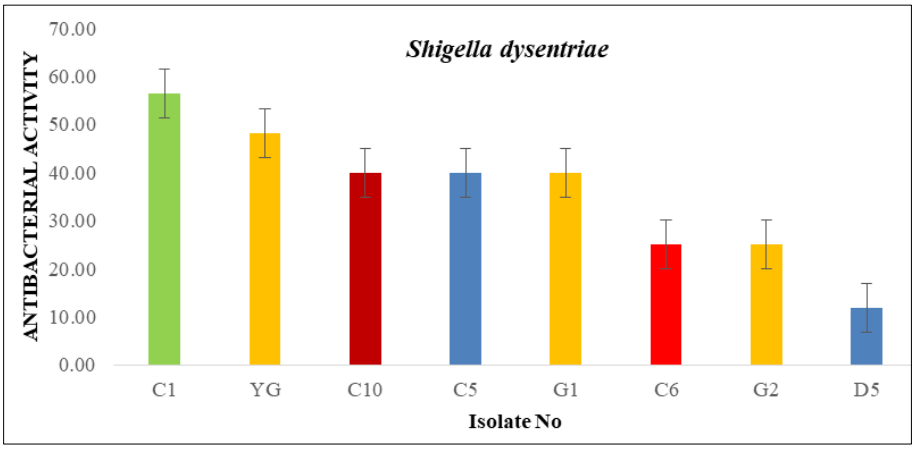

Fig 3: Antibacterial activity of *Lactobacillus* strain against *Shigella dysentriae*

4. Conclusion

Lactobacilli grown in MRS broth exhibit antibacterial activity against enteric pathogens, *E. coli*, *Salmonella* spp. and *Shigella dysentriae.* Among these three pathogens *E. coli* was found more susceptible followed by *Salmonella* spp. and *Shigella dysentriae.* This indicates that, these lactobacilli cultures can be grown and their antibacterial compounds can be isolated and used as potential preservatives against enteric pathogens in foods.

5. References

1. Amin JB, Singh RS, Chander H. Antibacterial property of *Lb. bulgaricus* WS against enteric pathogens. Asian Journal of Dairy Research. 1991;10:188-192.
2. Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, *et al*. Antimicrobial Activity of *Lactobacillus* Species against Carbapenem-Resistant Enterobacteriaceae. Frontiers in Microbiology. 2019;10:789.
3. Coman MM, Verdenelli MC, Cecchini C, Silvi S, Orpianesi C, Boyko N, *et al*. *In vitro* evaluation of antimicrobial activity of *Lactobacillus rhamnosus* IMC 501, *Lactobacillus paracasei* IMC 502 and SYNBIO against pathogens. Journal of Applied Microbiology. 2014;117:518-527.
4. De Man JC, Rogosa M, Sharpe ME. A medium for cultivation of lactobacilli. Journal of Applied Bacteriology. 1960;23:130.
5. Prbaha R. Studies on the antibacterial activity of *Lactobacillus acidophilus* cells for their incorporation into ice cream. M.Sc. Thesis. UAS Bangalore, 1984.
6. IS SP: 18. ISI Handbook of Food Analysis. Part XI, Dairy Products, Indian Standards Institution, Manak Bhavan, New Delhi, 1981.
7. Sarkar S, Kulia RK, Misra AK. Effect of incorporation of Gelodan SB253 and Nisin on the microbiological quality of Shrikhand. Indian Journal of Dairy Science. 1996;49:176-184.