

Antimicrobial Peptides-New Weapons Against Enteric Pathogens

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Abstract: Antimicrobial compounds produced by lactic acid bacteria [LAB] has broad spectrum of inhibition against pathogens and food spoilage microorganisms. LABS isolated from various fermented products as well as standard cultures were employed as test organisms against selected enteric pathogens. Pure bacteriocin eg, nisin was used as reference to compare with crude bacteriocin from natural sources. Metal chelator [EDTA] was used to enhance the bacteriocin activity against Gram-negative bacteria.

Key words: Lactic acid bacteria, domestic curd, commercial milk

Introduction

Reservoir of enteric pathogens in the environment contributes to disease burden and mortality in population. Enteric pathogens are predominating in sewage manur, polluted water used for irrigation purposes, thereby contaminating fresh produce (Puttalingamma *et al.*, 1985) Hence vegetables from field generally carry pathogens which multiply during storage and transport may contaminate food at different stages in the food chain causing health hazards. (Puttalingamma and Manja, 1998). It is universally accepted that there is no such thing as "Zero risk" food or drinks.

Antimicrobial properties of a few types of bacteria are of interest especially in biocontrol strategies for use in foods. In food preservation and safety, the indigenous microflora have advantages in suppressing undesirable microorganisms (Karen *et al.*, 2002). LAB cultures were used effectively against G+ve pathogens, *Coliforms*, *A.hydrophila*, *S.aureus*, *Bacillus cereus*, *Salmonella typhi*, and *Listeria monocytogenes* in ready to use vegetables. (Vescovo *et al.*, 1995). Antagonistic behavior of LAB is due to antimicrobial peptide production or the low molecular weight compounds produced. These bacteriocins produced by lactic acid bacteria have gained much attention as potentially useful food additives against food-borne pathogens. Class I bacteriocins (antibiotics) undergo extensive post-transnational modifications and contain unusual amino acids. Nisin, for instance, is a 34-residue peptide that is active against most Gram-positive bacteria. Nisin is the only bacteriocin available commercially and used worldwide since 1950 (Kelly *et al.*, 1998). Different bacteriocins exhibit different mode of action towards sensitive organisms. (Sarkar and Mishra, 2001). Incorporation of these substances in extending shelf life of vegetables and milk or its products has provided successful results (Balasubramanyam, 1995; Sarkar and Mishra, 2001). One of the oldest methods of

food preservation, which is still in practice is the process of fermentation. LAB was used as a bio preservative to control the growth of *Listeria monocytogenes* in fresh vegetables Kelly *et al.* (1998) Many predominant genera of LAB are available in natural habituates, which can be used in food preservation (Prabir and Sharmistra, 1996; Koterska *et al.*, 1998) LABS in combination are demonstrated to give better results. (Prabir and Sharmistra, 1996; Koterska *et al.*, 1998). LAB isolated from vegetables like radish was active against many food borne pathogens and undesirable spoilage bacteria in ready to eat products (Yildirm and Johnson, 1998). Considering the biopreservative effect of LAB it was proposed to screen a few species of LAB from natural habitat and study their antimicrobial activity against *Bacillus* spores. The present investigation was conducted to study the inhibitory activity of crude bacteriocin from *Lactococcus lactis sub ssp lactis*, *L. plantarum*, *Pediococcus acidilactis*, *Lactococcus cremoris* and nisin on commonly occurring enteric pathogens.

Materials and Methods

Isolation of LAB from natural source: Lactic acid bacteria were isolated from fermented products like curd, dose (Thick pan cake prepared from rice semolina and black gram paste) and idly (Fermented steam cooked pudding prepared from rice and black gram) batter both from domestic and commercial samples, collected from different parts of Mysore city. The samples were streaked on MRS agar plates and incubated under microaerophilic condition at 37°C for 48 hrs. The individual colonies were picked up with a sterile needle and transferred to MRS broth [Hi media, Mumbai, India] and incubated at 37°C and stored at 4°C.

Assay of antimicrobial activity: The well diffusion assay described by Tagg *et al.* (1976) was employed to

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Table 1: Antimicrobial activity of isolated LAB against *B. cereus* (Including well size)

Culture no	Source	Zone in mm
Std. Culture <i>L. lactis</i>	ATCC – 11454	30.7±0.404
<i>L. plantarum</i>	NCIM – 2084	24.8±0.583
<i>P. acidilactici</i>	NCIM – 2292	22.0±0.577
DFR - 1	Domestic curds	20.1±0.577
DFR - 5	Domestic curds	20.3±0.208
DFR - 10	Domestic Idli mix	20.3±0.057
DFR - 12	Domestic Idli mix	20.4±0.115
DFR - 14	Domestic curds	20.1±0.152
DFR - 20	Domestic dosa mix	20.2±0.057
DFR - 26	Domestic dosa mix	20.4±0.152
DFR - 27	Domestic dosa mix	20.5±0.1
DFR - 28	Domestic dosa mix	20.3±0.208
DFR - 29	Domestic curds	20.4±0.152
DFR - 30	Domestic curds	20.4±0.115
DFR - 31	Domestic Curds	20.5±0.115
DFR -32	Commercial dosa mix	20.2±0.152
DFR - 33	Commercial dosa mix	20.2±0.057
DFR - 34	Commercial dosa mix	20.9±0.115
DFR - 35	Commercial dosa mix	20.1±0.1
DFR - 36	Commercial Cheese	20.4±0.115
DFR - 56	Commercial Milk	21.2±0.115
DFR - 65	Commercial Curd	17.7±0.152
DFR - 67	Commercial Curd	20.1±0.208

5, 27, 31, 34, 36, showing high activity

Table 2: Variations in AMA exhibited by LAB isolates from selected food sources against *B.cereus*

Source	Mean area of the zone in cm		
		Maximum	Minemum
Curd	Range	17.7	20.5
	Mean	19.96 ± 0.01	
Idli	Range	20.3	20.4
	Mean	20.35 ± 0.01	
Dosa	Range	20.1	20.9
	Mean	20.5 ± 0.01	

12 samples each

assess the inhibitory activity against pathogenic organisms. Nutrient agar plates were seeded with test organisms (0.1%). The wells of 1mm diameter were made in the prepared plates using sterilized cork borer. The wells were filled with culture supernatant of lactic acid bacteria (0.2ml). Nisin (1000,ppm) was also used in one of the wells as reference standard. The plates were kept at 6°C for three hours, followed by incubation at 37°C for 18-20 hours. The results were compared with the inhibitory zones formed by a solution of the commercial preparation of nisin at 1000-ppm level. The diameter of the inhibitory zones formed were measured in mm.

Organisms used in the study: The cultures isolated from fermented products like curd, dosa and idli batter were used for the study standard culture of *L. lactics*, *L. plantarum*, *L. cremoris* and *P. acidilactici* obtained from ATCC and IMTECH, Chandigarh were used along with

the isolated cultures for comparisons. Pathogenes used were *S. typhi*, *Pseudomonas sp.*, *A. hydrophila*, *B. cereus*, *L. monocytogenes* and *S. aureus*.

Preparation of EDTA: EDTA (0.1 g) was pulverized and aseptically mixed with 2.0 ml of LAB suspension of 10^{10} cfu/ml viable cells.

Preparation of nisin: (Nisapline-brand, Apline and Barrette, Co, UK) 1000-ppm solution of nisin in 0.01 N HCl was adjusted at 4.0 pH.

Results and Discussion

LAB strains were isolated from different sources and their activity was tested against *B. cereus*-ATCC-11778 is presented in Table 1. The zone of inhibition varied from 17.7± 0.152 - 20.9±0.115 mm. Culture DFR- 34, isolated from commercial dosa mix showed highest activity (20.9 mm). 75 % (9/12) curds samples and 16.67 % idli batter and 66.7% dosa batter exhibited presence of LAB. The AMA activity of the isolated LAB cultures from the respective food samples varied from 17.3 to 20.3 as minimum and 20.4 to-20.9 as the maximum AMA activity indicating distribution of LAB in natural products. (Table. 2).

It is evident from Table 3 that nisin, the known bactericidal compound from lactic acid bacteria exhibited antagonistic effect against all the selected pathogens. The mean zone of inhibition varied from 30± 0.01 to 34± 0.01 mm. Maximum AMA was noticed with nisin since; this compound is a pure bacteriocin evidently exhibited higher effects than those observed with crude extracts of LAB. *L. lactis* strain is known to produce Nisin; our observations have indicated that, antagonistic effect seen with *L. lactis* was markedly higher than those from other strains of LAB. This confers the efficacy of the bacteriocin from *L. lactis* as potent antimicrobial agent. However, if the activity of *L. lactis* (crude extract) against food borne pathogens was compared to those of Nisin, Nisin exhibited considerably high activity. Undoubtedly, the differences in the activities may be due to the differences in purity of the bacteriocins. EDTA being metal chelator exerts an indirect effect on growth of microbes by limiting the availability of important metals. Addition of EDTA to nisin brought about considerably higher antagonistic effect than that seen with nisin alone. Research evidences also suggests that EDTA with crude bacteriocin or nisin effectively inhibit (likka and Tiina, 2000). The extent of effect as measured by the diameter of clear zone from the wells was high in *Pseudomonas* followed by *L. monocytogenes* i.e, 34.0 and 33.0 mm respectively. The diameter of the zones was least in case of *S.typhi*. Among the different strains of LAB, *L. plantarum* was noted to have the least effect since the zone diameter varied between 11.0 ± 0.1 mm to 22.0 ± 0.1 mm. Result suggests that extract from the

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Table 3: Inhibitory effect of nisin [1000 ppm] and selected strains of LAB on certain pathogens

Pathogens	Nisin	Nisin [EDTA]	<i>P. pentosaceus</i> [5X10 ⁹]	<i>L. lactis</i> [5X10 ⁸]	<i>L. plantarum</i> [5X10 ⁸]
<i>L. monocytogenes</i>	31±0.5	33±0.01	ND	ND	22±0.01
<i>Salmonella typhi</i>	20 ± 0.5	29±0.01	25 ± 0.01	30±0.01	11± 0.01
<i>Pseudomonas spp</i>	30 ± 0.1	34±0.01	30± 0.01	30±0.01	ND
<i>Staph aureus</i>	30 ± 0.1	32±0.01	ND	ND	ND
<i>A. hydrophila</i>	29± 0.5	30±0.01	26± 0.01	30± .01	22± 0.01

Table 4: Inhibitory effect of LAB in combination on selected pathogens

	<i>L. lactis</i> and <i>P. pentosaceus</i> LAB (10 ⁵)	<i>L. lactis</i> and <i>P. acidilactici</i> LAB (10 ⁵)
	Inhibitory zone in mm (dia ,Including well size)	
<i>Listeria monocytogenes</i>	32 ± 0.01	34.0±0.01
<i>Salmonella typhi</i>	25 ± 0.01	ND
<i>Pseudomonas spp</i>	31 ± 0.01	30.0±0.01
<i>Aeromonas hydrophila</i>	35 ± 0.01	35 ± 0.01
<i>B. cereus</i>	37 ± 0.01	29 ± 0.01
<i>Staph aureus</i>	14 ± 0.01	14 ± 0.01

Table 5: Antimicrobial activity of LAB and isolates [crude bacteriocins] against food borne pathogens (zone in mm, dia)

LAB	<i>B. cereus</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>A. hydrophila</i>	<i>Vibrio</i>
<i>L. lactis</i>	30.90 ±0.1	28.0 ± 0.58	30.0 ± 0.66	29.0 ± 0.50	13.2 ± 0.1
<i>L. plantarum</i>	24.8 ±0.2	21.6 ± 0.3	22.9 ± 0.57	22.6 ± 0.40	11.9 ± 0.05
<i>P. acidilactici</i>	22.0 ±0.5	28.0± 0.66	30.0 ± 0.50	29.9 ± 0.15	10.9 ± 0.25
<i>P. pentosaceus</i>	23.2±0.30	28.7±0.17	31.1 ± 0.0	30.0 ± 0.1	14.2 ±0.23
DFR-5	20.9 ± 0.5	18.9 ± 0.17	25.5 ± 0.5	22.1 ± 0.15	12.5 ± 0.6
DFR-27	29.8 ± 0.5	20.8 ± 0.17	ND	ND	ND
DFR-31	18.8 ± 0.5	12.8 ± 0.17	ND	16.9 ± 0.15	ND
DFR-34	26.2 ± 0.5	14.0± 0.17	17.8 ± 0.5	16.6 ± 0.15	12.8 ± 0.1
DFR-36	18.1±0.5	20.0±0.17	20.1±0.17	23.1±0.15	21.1±0.6

live cells occurring in the environment are as effective as nisin [bacteriocin isolated from LAB] in antagonizing pathogens.

A perusal of Table 4, suggests that a combination of LAB strains brings about an increased effect than that seen with single strain of LAB. *L. lactis* and *P. pentosaceus* exhibited higher AMA than that of *L. lactis* and *P. pentosaceus*, similarly *P. acidilactici* with *L. lactis* had a much higher zone of inhibition. It is possible that few strains are complementary to each other and exhibit promising results.

Table 5 presents the results about the antimicrobial activity of standard cultures of LAB against the selected pathogens as well as natural isolates obtained from food sources. It is encouraging to note that, all the strains of LAB investigated exhibit antagonistic effect against pathogens except for *Vibrio*. The most potent being *L. lactis*, which had the maximum antagonistic effect with relatively high inhibitory zones (28.0 for *S. aureus* and 30.9 mm for *B. cereus*). Other workers have also demonstrated a higher activity in *L. lactis*. Maximum inhibitory activity was shown by *L. lactis* against *L. monocytogenes*, *B. cereus* and *A. hydrophila* (30.0, 30.0 and 29.0mm). *L. plantarum* showed variations in the zone of inhibition against different pathogens used, diameter of zones ranged from 11.9 to 24.8 mm. *P.*

acidilactici had less inhibitory activity against *Vibrio* (10.9 mm) while maximum activity was shown against *L. monocytogenes* (30.0 mm), *A. hydrophila* (20.9 mm), *S. aureus* (28.0 mm) and *B. cereus* (22.0 mm). The LAB strains was effective in inhibiting the selected pathogens, however low activity was seen against vibrio, since the zone diameter ranged between 10.9 ± 0.25 to 14.2 ± 0.23 mm as against 22 ± 0.5 to 30.9± 0.1 for other pathogens. A similar trend was noted with the natural isolates. The exception was the DFR 36, which exhibited markedly higher AMA against vibrio. (21.1± 0.6 mm).

Conclusion: The present work brings to light the possibility of including LAB cultures in processing of foods to effectively reduce the contamination, increase the shelf life of vegetables or any other food as well as control of infections from food borne pathogens. Such usage has the additional benefit since LAB is commercially used organisms in food, time has trusted its efficacy as food ingredients in curds. It is evident that nisin is definitely a potent bactericidal agent having more or less similar effect on the selected pathogens. The bactericidal activity of the intact organisms varied with differences in the strain of LAB. Most of the LAB strains exhibited inhibition to *L. monocytogenes*, *B. cereus*, *S. aureus*, *A. hydrophila* and to a lesser extent against *Vibrio* species.

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