ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIAL ISOLATES

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ABSTRACT

Micro-organisms have tendency to produce antimicrobial substances which show biological activity against other kind of micro-organisms. This phenomenon of bacterial antagonism is observed in lactic acid bacteria with competitive advantages. The lactic acid bacteria are commonly present in many fermented products, fruits and milk products. The variety of antimicrobial substances produced by lactic acid bacteria showing good inhibition capacity include production of lactic acid, acetic acid, hydrogen peroxide, carbon dioxide, diacetyl and bacteriocin. Bacteriocins produced by lactic acid bacteria are the subject of intense research because of their antimicrobial activity against food born bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium botulinum* and several others. Bacteriocins may be bacteriostatic or bactericidal with narrow or broad range of activity. The main of the study was to study the antimicrobial activity of such lactic acid bacterial isolates.

Keywords: Bacteriocin, Lactic acid bacteria, Antimicrobial activity, Antagonistic activity.

INTRODUCTION

Bacteriocins are accepted as heterogeneous group of proteinaceous compounds varying in spectrum of activity, mode of action, molecular weight, generic origin and biochemical properties. Since 1969, considerable research has been continuing on bacteriocins from lactic acid bacteria to derive optimum human benefits. They are also effective in the treatment of variety of disorders including colitis, prevention of decolonization of the intestine with the pathogens after antibiotic treatment, the gastroenteritis and many more. Lactic acid bacteria have natural habitat in many foods as well as in intestinal tract of human and animals¹. The majority of sources from which lactic acid bacteria can be isolated are plant materials including flowers, leaves, fruits etc. The main application of this natural bacterial phenomenon is in production of antimicrobial substances by one kind of microorganism, which shows biological activity against other type of microorganisms². These lactic acid bacteria either by producing higher acidic conditions utilizing sugars and carbohydrates present in surrounding environment and forming lactic acid create unfavorable conditions for growth of other microorganisms by lowering pH or by producing antimicrobial substances like bacteriocin, compete successfully with other microorganisms³. Antimicrobial compounds produced by lactic acid bacteria made them best choice for use them as probiotics as they not only improve microbial safety of food product but they are as natural inhabitant of gut environment improve their stability and viability in gut after consumption⁴. The possible mode of action of lactic acid bacteria are suppression of growth of unwanted microorganisms by production of antimicrobial compounds by competing with them for nutrients and adhesion sites, by alteration of microbial metabolites and stimulation of immunity etc⁵. The genera of lactic acid bacteria includes those members of family *Lacto bacillaceae* most commonly encountered in food and dairy product, in mouth and intestinal tract of human and animals and vegetable materials. Lactic acid bacteria are Gram positive non sporulating cocci or rods dividing in one plane only (except *Pediococci*), catalase negative and benzidine test

negative (some strains possess pseudocatalase activity) usually non motile, obligate fermenters, producing lactic acid and sometimes volatile acids and CO₂. This antimicrobial activity of lactic acid bacteria has created the interest to study lactic acid bacteria as potent antimicrobial substances producer. Each of the antimicrobial compounds, produced by lactic acid bacteria especially in combination, can be used to extend the shelf life and safety of food products⁶. Bacteriocins produced by lactic acid bacteria are subject of intense research because of their antimicrobial activity against food born bacteria such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium botulinum* and several others⁷. The local lactic acid bacteria may have such antimicrobial potential and hence they must be isolated and screened for the purpose.

Aims and Objectives

The main objective of project work is to study antimicrobial and antagonistic activity of lactic acid bacterial isolates, individually and in combination, isolated from local area from various sources like food material and food preparations. In the view of above background a project was undertaken with following aims and objectives:

- **Isolation of Lactic acid bacteria**
- **Antimicrobial activity studies**
- **Isolation of Lactic acid bacteria**
 1. Collection of material for lactic acid bacteria isolates.
 2. Isolation of lactic acid bacteria from different sources using different nutrient media.
 3. Identification of lactic acid bacterial isolates.
- **Antimicrobial activity studies**
 1. Screening of bacteriocin producing isolates.
 2. Effect of bacteriocin preparations individually on lactic acid bacterial isolates.
 3. Effect of bacteriocin preparations individually on pathogenic organism.
 4. Effect of bacteriocin preparations in combination on different organism.

MATERIALS AND METHODS

Materials

Number of fruits and vegetables, milk products and fermented foods were collected from local areas for isolation of lactic acid bacteria. Different media were used for enrichment of lactic acid bacteria like *Lactobacillus* selection medium for *Lactobacillus*, MRS broth for *Lactobacilli* and *Streptococci*, Acetyl Broth for *Pediococcus* and *Leuconostoc*, Lactic Broth for yoghurt bacteria (*Streptococci*). For antimicrobial studies against pathogenic organisms, types of pathogenic organisms used were *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsella pneumonia*.

Methods

Sample collection

Ten food samples were collected from different places in autoclaved polythene bags and they included fruits, milk, milk products food and fermented products.

Enrichment

For lactic acid bacteria, various samples were diluted (1:10) in sterile saline water and 1 ml aliquots were added in different media broth and kept for 1-2 days for enrichment at room temperature (28-30°C)

Isolation of lactic acid bacteria

A loopful from each of enriched media were streaked on respective agar medium and incubated for 1 to 2 days at 30°C under micro-aerophilic conditions in anaerobic jars.

Characterization of group of lactic acid bacteria

Morphological Observation⁸

Isolated colonies were suspended in sterile saline and were Gram stained to observe morphology.

Catalase test

Catalase test was carried out by lifting some part of colony with sterile glass rod and dipping in tube containing 2 ml H₂O₂ (30 % v/v) solution and observed for gas evolution (effervescence).

Oxidase test

Oxidase test was performed by rubbing some part of colony on filter paper soaked in reagent N-N-N'-N'' tetra methyl p-phenylene diamine dihydrochloride solution (1 %) and observed for blue colour development.

Benzidine test

Benzidine hydrochloride solution was prepared upto saturation in acetic acid. The filter paper was soaked in saturated solution of benzidine hydrochloride in acetic acid. Then part of colony rubbed on filter paper with sterile glass rods and few drops of H₂O₂ were added on it and development of deep purple colour was taken as indication of positive test.

Glucose fermentation

For glucose fermentation studies, peptone broth with glucose, pH indicator (Bromothymol Blue) and Durham's tube (for gas detection) was inoculated with loopful culture from each isolate and incubated at 37°C for 24-28 h.

Screening of lactic acid bacterial isolates for antagonistic property

Antagonistic activity of lactic acid bacterial isolates was studied against themselves and also against few Gram positive and Gram negative pathogenic bacteria.⁹ Each isolate was grown on MRS broth at optimum conditions (at R.T. for 48 h) and after incubation and aseptic centrifugation the broth supernatant was used for qualitative determination of antimicrobial activity by:

1. Agar gel diffusion method (Ditch method)
2. Agar gel diffusion assay (Well method)

In these methods, culture of test organism was spread on the medium or streaked around well or ditch, respectively and in well or ditch supernatant was added on MRS agar plates. After incubation (30°C for 24-28 h) zones of inhibition of test organisms were observed.

RESULTS

Isolation and characterization of lactic acid bacteria

Using ten different samples which were enriched and then grown on different media, 35 different lactic acid bacterial isolates were obtained which were further characterized as shown in Table 1, 2 and 3.

Antimicrobial studies on lactic acid bacterial isolates

Antimicrobial activity studies were carried out amongst isolates themselves and against pathogenic organisms, using preparations of bacteriocin individually or in combination with Gel diffusion method (Ditch method or Well method) for qualitative determinations. By using 10 different lactic acid bacterial isolates 1, 4, 7, 9, 15, 18, 21, 26, 32 and 34 (selected randomly) as indicator test organisms and bacteriocin preparation, antimicrobial effect of isolates were studied. The results are tabulated in the Table 4, 5, 6 and 7.

DISCUSSIONS

As per the Tables 1, 2 and 3 all isolates were belonging to group lactic acid bacteria as they showed catalase (except few), oxidase and benzidine hydrochloride tests negative. Morphological observations revealed that these organisms were Gram positive cocci and rods showing similarities with species of group lactic acid bacteria. Out of 35 isolates 25 were homofermentative and 10 were heterofermentative. Out of 35 isolates 18 were rod shaped (*Lacto bacilli*) and these *Lacto bacilli* 13 were rod shaped (*Lacto bacilli*) and these 8 were homofermentative and 5 were heterofermentative. In these isolates 19 were homofermentative cocci (*Lactic streptococci*), 3 were homofermentative cocci in packets (*Pedio cocci*) while remaining were heterofermentative cocci in chains (*Leuconostoc* spp.). As per results out of 35 isolates, 6 isolates namely isolate No. 1, 7, 9, 15, 21 and 32 showed good spectrum of antimicrobial activity, For studying antimicrobial action of bacteriocins in combination, these preparations from each isolate were mixed with each other in different combinations using equal quantities of each preparation. The inhibition spectrum of bacteriocin preparations when used in combination was seemed to be more effective than using them individually (Table 7). The antimicrobial activity is observed maximum i.e. zone of inhibition is 7 mm for the combination of curd and apple isolate whereas it is 4 mm when curd isolate is used individually. When curd isolate is combined with butter and milk isolate it is showing better results than the individual curd isolate. The same results are followed by all the isolates.

Table 1: Lactic Acid Bacterial Isolates obtained from different samples

Samples	LAB Isolate number
Curd	1, 2, 3, 4
Idli flour	5, 6, 7, 8
Milk	9, 10, 11
Cheese	12, 13, 14
Butter	15, 16, 17, 18
Apple	19, 20, 21
Guava	22, 23, 24
Chiku	25, 26, 27, 28
Banana	29, 30, 31, 32
Wheat flour	33, 34, 35
Total	35

LAB- Lactic Acid Bacteria

Table 2: Characterization of Isolates of Lactic Acid Bacteria

Isolate No.	Gram staining	Catalase	Oxidase	Benzidine	Glucose Fermentation
1	Gram positive diplococci	-	-	-	Acid
2	Gram positive cocci in packets	-	-	-	Acid
3	Gram positive rods	-	-	-	Acid
4	Gram positive cocci in packets	-	-	-	Acid
5	Gram positive rods	-	-	-	Acid
6	Gram positive cocci	+	-	-	Acid
7	Gram positive short rods	+	-	-	Acid
8	Gram positive cocci in pair	-	-	-	Acid
9	Gram positive rods	+	-	-	Acid
10	Gram positive cocci	+	-	-	Acid
11	Gram positive rods	-	-	-	Acid
12	Gram positive rods	+	-	-	Acid and Gas
13	Gram positive ovoid rods	+	-	-	Acid
14	Gram positive cocci	-	-	-	Acid and Gas
15	Gram positive cocci	-	-	-	Acid and Gas
16	Gram positive rods	-	-	-	Acid and Gas
17	Gram positive cocci	-	-	-	Acid
18	Gram positive cocci	-	-	-	Acid and Gas
19	Gram positive rods	-	-	-	Acid
20	Gram positive rods	-	-	-	Acid
21	Gram positive rods	-	-	-	Acid
22	Gram positive chains of cocci	-	-	-	Acid and Gas
23	Gram positive cocci	-	-	-	Acid and Gas
24	Gram positive cocci	-	-	-	Acid and Gas
25	Gram positive rods	-	-	-	Acid
26	Gram positive rods	+	-	-	Acid
27	Gram positive cocci	+	-	-	Acid
28	Gram positive rods	-	-	-	Acid
29	Gram positive cocci	-	-	-	Acid
30	Gram positive rods	-	-	-	Acid
31	Gram positive rods	-	-	-	Acid
32	Gram positive rods	-	-	-	Acid
33	Gram positive ovoid cocci	-	-	-	Acid
34	Gram positive cocci in packets	-	-	-	Acid and Gas
35	Gram positive long rods	-	-	-	Acid and Gas

Table 3: Tentative Identification of Lactic Acid Bacterial Isolates

Morphology	Fermentative Pattern	Tentative Identification
Gram positive cocci	Acid	<i>Lactic streptococci spp.</i>
Gram positive cocci	Acid	<i>Leuconostoc</i>
Gram positive cocci in packets	Acid and Gas	<i>Pediococcus</i>
Gram positive rods	Acid	Homofermentative <i>Lactobacillus spp.</i>
Gram positive rods	Acid and Gas	Heterofermentative <i>Lactobacillus spp.</i>

Table 4: Antimicrobial Activity of Bacteriocin Preparation against LAB Isolates with Gel diffusion Assay by Ditch Method

Bacteriocin Preparation No.	Isolate No. used as test organism									
	1	4	7	9	15	18	21	26	32	34
1	+	-	-	+	-	-	-	-	+	-
2	+	-	-	-	-	-	-	+	-	-
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-
5	-	+	-	-	-	-	-	-	-	-
6	-	+	-	+	-	-	+	-	-	+
7	+	-	+	-	-	-	+	-	+	-
8	+	-	+	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	+	-	-	-	-	-
11	-	-	-	-	+	-	-	-	+	-
12	-	-	-	-	-	-	-	+	-	-
13	-	+	-	-	-	+	-	-	-	-
14	-	-	-	+	-	+	-	-	-	+
15	+	+	-	+	-	-	-	+	-	-
16	+	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-

Bacteriocin Preparation No.	Isolate No. used as test organism									
	1	4	7	9	15	18	21	26	32	34
20	-	-	-	-	-	+	-	+	-	-
21	+	-	-	+	-	-	-	-	-	-
22	-	-	-	+	-	-	-	-	+	+
23	+	-	+	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-
25	-	-	-	+	-	-	-	-	-	-
26	-	-	-	-	-	-	+	-	-	-
27	-	+	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	+	+	-
29	-	-	-	-	-	+	+	-	-	-
30	-	-	-	-	-	+	-	-	-	+
31	+	-	-	-	+	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-
33	-	+	-	-	-	-	-	-	-	-
34	-	-	-	-	+	-	-	-	-	+
35	-	+	-	-	+	-	-	-	-	-

+ = Sensitive

- = Resistant

Table 5: Antimicrobial Activity of Bacteriocin Preparation against Pathogenic Organisms

Bacteriocin preparation from isolate no.	Test organisms used			
	<i>Staphylococcus aureus</i>	<i>Klebsella pneumonia</i>	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>
1	+	-	+	+
7	+	-	-	-
9	+	-	-	-
15	+	-	+	-
21	+	+	+	-
32	+	+	-	+

+ = Sensitive

- = Resistant

Table 6: Antimicrobial Activity of Bacteriocin used Individually using Isolate no. 8 used as Test Organism

Bacteriocin preparation from isolate no.	Zone of inhibition (mm)
1(Curd)	4
7 (Idli Batter)	3
9 (Milk)	6
15 (Butter)	2
21 (Apple)	4
32 (Banana)	3

Table 7: Antimicrobial Activity of bacteriocin in Combination using Isolates no. 8 as Test Organism

Bacteriocin preparation in combination	Zone of inhibition (mm)
1 and 7	5
7 and 9	4
9 and 15	2
15 and 21	4
21 and 32	3
32 and 1	4
1 and 15	5
1 and 21	7
1 and 9	5
7 and 15	4
7 and 21	2
7 and 32	3
9 and 15	4
9 and 21	4
15 and 32	3
9 and 32	4

The bacterial isolate no. 1 showed broad spectrum of antagonism against pathogens, both Gram positive and Gram negative ones. *Staphylococcus aureus* strain showed maximum sensitivity; followed by *Pseudomonas aeruginosa*, *Klebsella pneumonia* and *E. coli*.

CONCLUSION

Ten different samples were used as source of lactic acid bacteria and using different media, 35 different lactic acid bacterial isolates were obtained which contained homo and heterofermentative *Lacto bacilli*. Homofermentative cocci (*Lactic streptococci* and *Pedio cocci*) and heterofermentative cocci (*Leuconostoc* spp.) and two laboratory lactic acid bacterial isolates were obtained. All the isolates were grown in MRS broth and bacteriocin preparations were made and tested against 10 randomly selected lactic acid bacterial isolates out of 10 isolates, isolate no. 8 showed maximum sensitivity spectrum. Out of 35 isolates six isolates viz. isolate no. No. 1, 7, 9, 15, 21 and 32 showed maximum antimicrobial spectrum against test pathogens. In the combination studies using bacteriocin preparations of above six isolates it was found that better results were obtained in combination than individual bacteriocin activity. These studies further showed that out of above six isolates, the isolate no. 1 was most promising in the lot regarding antimicrobial activity and hence it was then used for further production and optimization studies.

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REFERENCES

1. Jay J.M. Modern food microbiology, fourth edition, CBS publishers and distributors; 1966.
2. Luc De Vuyst, Frederic Roy. Bacteriocins from Lactic acid Bacteria: production, purification and food application Journal of Molecular Microbiology and biotechnology 2007; 13: 194-199. <http://dx.doi.org/10.1159/000104752> PMID:17827969
3. Adentuji VO, GO Adegoke. Bacteriocin and cellulose production by Lactic acid bacteria isolated from West African soft cheese. African Journal of biotechnology 2007; 2616-2619.
4. Yang Rongguang, Yanling Chen and Bibek Ray. Enhanced bacteriocin production by lactic acid bacteria in a dairy based medium, supplemented with B galactosidase. Indian Journal of Microbiology 1999; 39: 245-250.
5. Havenaar R and Huis J. Probiotics: A general view. London Elsevier Appl. Sci 1992; 209-224. http://dx.doi.org/10.1007/978-94-011-2364-8_9
6. Deshmukh AM. Handbook of Media Stains and reagents in Microbiology PAMA Publications; 1996.
7. Coconnier MH, Bernet MF, Kernis S Chauviere G. Inhibition of adhesion of entero invasive pathogens to human intestinal Caco-2 cells by *Lactobacillus acidophilus* FEMS Microbiology Letters 1993; 10: 299-305. <http://dx.doi.org/10.1111/j.1574-6968.1993.tb06339.x>
8. Strompfova V, Laukova A, Mudronova D. Effect of bacteriocin like substances produced by *Enterococcus faecium* EF55 on the composition of Avian Gastrointestinal Microflora. ACTA VET BRNO 2003; 559-564.
9. Joshi H, Choudhary BL. *In vitro* inhibition of toxic bacteria by bacteriocins of Lactobacillus. J of Her Med and Toxicology 2007; 55-57.

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