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Screening of inhibitory activity of lactic acid bacteria from fresh meat

S Aarthi Nishanthi Mary, * V Sumathi

Department of Microbiology, Annamalai University, Tamil Nadu, India

Abstract
Aim: Screening of inhibitory activity of lactic acid bacteria from fresh meat to identified.
Introduction: In the present study, Lactic Acid Bacteria (LAB) was isolated from fresh meat by using MRS and M17 medium. Totally 80 LAB was isolated and identified as *Lactobacillus spp.*, *Pediococcus spp.*, and *Lactococcus sp.*
Materials Methods: From different LAB isolates, *Lactobacillus spp*. was found relatively dominating species of fresh meat. Ten LAB were selected and screened for their inhibitory activity against the indicator food pathogenic strains such as.
Results: *Proteus spp., Staphylococcus aureus, Klebsiella spp., Listeria monocytogenes,* and *Salmonella spp*., estimated by well diffusion and disc diffusion method. Among the ten LAB strains *Lactobacillus sp.*, gave highest zone of inhibition against five food pathogens.

Keywords: lactic acid bacteria, fresh meat, *lactobacillus sp*

Introduction

Meat is highly regarded in many parts of the world as nutritious and valuable qualities of protein, vitamins, fat, essential minerals, B vitamins and iron [1]. Meats are highly perishable foods which provide excellent source for growth of many hazardous microorganisms that can cause infection in human and spoilage of meat and economic loss [2]. Several methods have been used to preserve including cooking, fermenting, salting, smoking, for preservation of meat and meat products. Fresh meat has lower quality of microbial contamination and it does shorten shelf life [3].

Lactic acid bacteria are part of the initial micro biota, typically mesophilic, which can grow easily at 5- 45° C, under aerobic, anaerobic or micro aerobic terms. LAB is gram positive bacteria, non spore forming, Coccobacilli or rod shaped, Oxidase and Catalase negative. Many genera of bacteria produce lactic acid as a primary or secondary end product of fermentation. The most important role of lactic acid bacteria is its protective role against infections and colonization of pathogenic microorganisms in the digestive track. LAB culture must compete with the relatively high indigenous microbial loads of raw meat, to actively inhibit pathogenic and spoilage bacteria [4-5]. LAB is most important group of microorganisms used in food fermentation include some species of the genera *Lactobacillus, Lactococcus, Streptococcus, Leuconostoc,* and *Pediococcus* [6]. Some LAB exhibit potent antimicrobial activities in the form of small, heat stable, antimicrobial peptides called Bacteriocins [7-8]. LAB exert strong antagonistic activity against many microbes including food spoilage organisms and pathogens by producing various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bacterial peptides during lactic acid fermentation [9].

Bacteriocins are antimicrobial proteinaceous compounds that are inhibitory towards sensitive strains and are produced by both gram positive and gram negative bacteria [10]. The inhibitory spectrum of some bacteriocin includes food spoilage and food pathogenic microorganisms [11].

Materials and methods

Collection of Sample

Fresh meat sample was purchased from Chidambaram market. The sample were collected and immediately transported to laboratory under cool condition for LAB isolation. Food borne pathogens used for testing antibacterial activity were *Proteus sp., Staphylococcus aureus, Klebsiella spp., Listeria monocytogenes* and *Salmonella spp.,* the strains obtained from MTCC.

Isolation and identification of lactic acid bacteria

LAB were isolated from fresh meat by adding 10 gm of meat sample and mixed with 90 ml of normal saline solution (8.5 gm Nacl/L) and homogenizing for 2 min [12]. Serial dilutions up to10^{-7} were prepared and appropriate dilutions were plate onto De Man Rogosa and Sharpe agar. Duplicate plates were incubated at 37° C for 24 hours. After growing a single colony was tested and examined morphologically and microscopically for purity and then subculture in MRS and M17 broth [13-14].

Preparation of cell free supernantants

Ten ml of MRS broth with LAB and incubated at 30° C for 48 hrs. After incubation, bacterial cells were removed by centrifuging the culture at 6000 rpm for 15 min at 4° C. The pH values of supernatant were adjusted to pH 6.5-7.0 by the addition of 1 N NaOH, filtration of the supernatant through 0.2 mm pore size filter thus obtaining cell free filtrate [13].

Antibacterial activity

Well diffusion method

The antibacterial activity of the isolated LAB (cell free filtrate) against the pathogenic bacteria (*Proteus spp., Staphylococcus aureus, Klebsiella spp., Listeria monocytogenes* and *Salmonella spp.,*). The pathogenic test bacteria were incubated in Nutrient broth at appropriate temperature for 24 hrs. Petri dishes containing 20 ml of Muller Hinton agar were prepared previously and inoculated

with 0.1 ml of 24 hrs broth culture of pathogenic bacteria. Once solidified the dishes were stored for 2 hrs in a refrigerator. Five wells were made and filled using different concentration like 5µl, 10µl, 15µl, 20µl, 25µl of cell-free filtrate and the petri dishes were incubated at 37 °C for 24 hrs. Then the diameter of the inhibition zone was measured with callipers in mm. The antibacterial activity was determined by measuring the clear zone around the colonies.

Disc diffusion method

Paper disc assay was adopted for evaluation of antibacterial activity of LAB (cell free filtrate). The surface of a plate containing Mueller Hinton agar (Hi media) was swabbed with tryptic soy broth containing indicator strains of *Proteus spp., Staphylococcus aureus, Klebsiella spp., Listeria monocytogenes* and *Salmonella spp.,* were prepared separately. The seeded medium was poured in sterile petriplate and the selected was prepared in different concentrations. Paper disc dipped in sterile water served as control. Different concentrations like 5µl, 10µl, 15µl, 20µl, 25µl were added in paper disc and incubated for 24hrs at 37ºC. After incubation period the diameter of inhibition zone formed around the paper disc were measured in mm.

Results

Totally 80 lactic acid bacterial strains were isolated from fresh meat. The identified genus were, *Lactobacillus spp., Pediococcus spp., and Lactococcus sp. Lactobacillus spp.,* was found relatively dominating species of Meat. The isolated Lactic Acid Bacteria (LAB) from fresh meat on MRS and M17 media shows positive for Gram strain, negative for indole, catalase, motility and spore forming with rod shape LAB particularly those belonging to beneficial and non-pathogenic bacteria. The fresh meat and fresh meat products are susceptible to biochemical changes due to the microbial growth. Among the 80 isolates of 10 LAB were tested against five food pathogens by well diffusion assay and disc diffusion method was used to measure inhibition zone. The cell free filtrate of ten isolated LAB gave zone of inhibition against the indicator food pathogenic strains such as *Proteus spp., Staphylococcus aureus, Klebsiella spp., Listeria monocytogenes,* and *Salmonella spp.*,

Among the ten LAB, the strongest diameter zones of (14-16 mm) obtained with the extracts of *Lb- 2, Lb- 3,* and *Lb- 5* against *Proteus spp., Staphylococcus aureus, Klebsiella spp., Listeria monocytogenes,* and *Salmonella spp.* Smallest or weak diameter zones (6-9 mm) of LAB are *Pc- 1* and *Lc-1.* The intermediate diameter zones (10-13 mm) of LAB *Lb- 6* and *Lb- 4and the* resistant pathogenic growth was obtained with the extracts of *Lb- 1, Pc- 2 and Pc- 3* (Table 1 to Table 5). The strains of LAB which showed the largest zone *viz., Lb- 2, Lb- 3,* and *Lb- 5,* of growth inhibition was selected for further strain development studies. Ten lactic acid bacteria were selected and allowed for antibacterial activity against five different bacterial pathogens which are usually present in food and can cause food borne illness in human being. All LAB were subjected to inhibitory activity test using agar well diffusion method and disc diffusion method. LAB having different spectra of action and isolated from different environments it's worthwhile. According to Klaenhammer, 99% of all bacteria may make at least one bacteriocin.

Table 1: Screening of antibacterial activity of LAB against *Proteus spp*

S. No	Lactic acid bacteria	Diameter of inhibitory zone	
		Well diffusion method (mm)	Disc diffusion method(mm)
1	Lb- 1	-	-
2	Lb- 2	15.2	14.5
3	Lb- 3	14.8	14.3
4	Lb- 4	11.6	11.2
5	Lb- 5	14.5	14
6	Lb- 6	12.7	12.5
7	Pc- 1	7.8	7.5
8	Pc- 2	-	-
9	Pc- 3	-	-
10	Lc- 1	6.9	6.5

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Table 2: Screening of antibacterial activity of LAB against *Staphylococcus aureus*

S. No	Lactic acid bacteria	Diameter of inhibitory zone	
		Well diffusion method (mm)	Disc diffusion method(mm)
1	Lb- 1	-	-
2	Lb- 2	15.1	14.7
3	Lb- 3	14.6	14.5
4	Lb- 4	11.6	11.2
5	Lb- 5	14.4	14.5
6	Lb- 6	12.5	12.2
7	Pc- 1	7.8	7.5
8	Pc- 2	-	-
9	Pc- 3	-	-
10	Lc- 1	6.8	6.3

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Table 3: Screening of antibacterial activity of LAB against *Klebsiella spp*

S. No	Lactic acid bacteria	Diameter of inhibitory zone	
		Well diffusion method (mm)	Disc diffusion method (mm)
1	*Lb- 1*	-	-
2	*Lb- 2*	14.8	14.5
3	*Lb- 3*	14.6	14.3
4	*Lb- 4*	10.4	10.6
5	*Lb- 5*	14.5	14.3
6	*Lb- 6*	10.5	10.3
7	*Pc- 1*	6.2	6.5
8	*Pc- 2*	-	-
9	*Pc- 3*	-	-
10	*Lc- 1*	6.1	6.3

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Table 4: Screening of antibacterial activity of LAB against *Listeria monocytogenes*

S. No	Lactic acid bacteria	Diameter of inhibitory zone	
		Well diffusion method (mm)	Disc diffusion method (mm)
1	Lb- 1	-	-
2	Lb- 2	15.1	15.2
3	Lb- 3	14.9	15
4	Lb- 4	10.6	10.8
5	Lb- 5	14.7	14.5
6	Lb- 6	11.3	10.8
7	Pc- 1	7.9	8
8	Pc- 2	-	-
9	Pc- 3	-	-
10	Lc-1	7.4	7.8

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Table 5: screening of antimicrobial activity of LAB against *Salmonella spp*

S.NO	Lactic acid bacteria	Diameter of inhibitory zone	
		Well diffusion method (mm)	Disc diffusion method (mm)
1	Lb- 1	-	-
2	Lb- 2	15.3	15
3	Lb- 3	14.7	14.6
4	Lb- 4	10.3	10.1
5	Lb- 5	14.3	14
6	Lb- 6	10.5	10.2
7	Pc- 1	7.3	6.8
8	Pc- 2	-	-
9	Pc- 3	-	-
10	Lc-1	6.5	6

Diameter of the inhibition zone: weak (6-9 mm), intermediate (10-13 mm), strong (14-16 mm), no growth (-)

Discussion

Certain strains of lactobacilli are more commonly isolated from meats [19]. Isolation been began with an enrichment so as to increase the initial biomass and to give a better chance to detect lactic acid bacteria [20].Totally 80 lactic acid bacterial strains were isolated from fresh meat. The identified genuses were *Lactobacillus spp., Pediococcus spp., and Lactococcus sp. Lactobacillus spp.,* was found relatively dominating species of Meat. The presence of such bacteria has been reported in earlier studies [18] these characters were according to [16]. This revealed that the LAB inhibited all the pathogenic bacteria according to mentioned that inhibition was scored positive and width of the clear zone around the colonies [19]. Lactobacillus is important in food fermentation as well as the production of bacteriocin [15]. Lactic acid bacteria originally isolated from fresh meat products are probably the best candidates for improving the microbiologically safety of these foods, because they are well adapted to the conditions of meats and should be more competitive than Lactic acid bacteria from other sources [19]. Many studies like bacterial assay [21] were carried out in Nigeria [14], using poultry meat to isolate LAB [22, 19] for their antibacterial activity against several microorganisms.

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