15 – Antimicrobial activity of local strains of lactococcus spp. and their supernatants tooards pathogenic bacteria

ANTIMICROBIAL ACTIVITY OF LOCAL STRAINS OF *LACTOCOCCUS* SPP. AND THEIR SUPERNATANTS TOWARDS PATHOGENIC BACTERIA

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ABSTRACT

Five local strains of lactic acid bacteria (LAB) as *Streptococcus thermophilus; Lactococcus lactis* subsp. *lactis; L. lactis* subsp. *cremoris; L. lactis* subsp. *diacetilactis* and *Leuconostoc* spp. were used for their antimicrobial activity as single or mixed cultures or mixed with five types of antibiotics towards seven genera of pathogenic bacteria. From the obtained results; mixture of *L. diacetilactis* with *Leuconostoc* sp. gave best activity followed by mixed culture of *L. lactis* and *Leuconostoc* sp. The statistical analysis showed that there were significant differences at 0.01 & 0.05 levels in MIC of LAB cells and supernatants mixed with antibiotics against some pathogenic bacteria.

الفعالية الميكروبية لعتر محلية من بكتريا حامض اللاكتيك الكروية وروا شحها ضد البكتريا المرضية

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المستخلص

استخدمت خمس عتر لبكتريا حامض اللاكتيك الكروية شـــملت *Streptococcus thermophilus* و *Lactococcus lactis* subsp. *Lactis* و subsp. *Cremoris* لـ *L. lactis* و subsp. *diacetilactis* لـ *L. lactis* و *Leuconostoc* sp. لقياس فعاليتها الحيويـــة كبكتريا مفردة أو كمزارع خليطه أو خلطها مع خمس أنواع من المضادات الحياتية ضد سبعة أنواع من البكتريا المرضيـــة. مـــن النتـــائج المستحصل عليها وجد أن خليط المزارع المؤلف من *L. diacetilactis* و *Leuconostoc* sp. له القابلية المثلى ضد البكتريا المرضيـــة ويأتي من بعدة خليط المزارع *L. lactis* و*Leuconostoc* sp. ووجد أن هناك فرق معنوي وخصوصا عند استخدام خلايا أو را شح بكتريا حامض اللاكتيك مع المضادات الحيوية.

INTRODUCTION

Among lactic acid bacteria (LAB), lactococci are the main components of the mesophilic starter cultures used in the manufacture of most dairy products. They contribute to the development of sensorial properties of fermented products and prevent the growth of food – borne pathogenic and food – spoilage organisms (8).

Their antagonistic effect relies mainly on lactic acid excretion, but also on other antimicrobial compounds, such as bacteriocins (4), acetaldehyde, diacetyl, hydrogen peroxide, organic acid and carbon dioxide (7).

Antimicrobial compounds produced by (LAB) have provided these organisms with a competitive advantage over other microorganisms. Exploitation of antibiosis of (LAB) is the best choice not only for improving the microbial safety of the food products but as probiotic preparations, because of their natural a deputation to the gut environment. Probiotics need to be acid tolerant bacteria and exhibit resistance to lysozyme present in the saliva and other enzymes, gastric juice and duodenal fluids. Many (LAB) are resistant to the bile salt present in the gut and survive the intestinal motility and adhere well to gastric mucosa (13).

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The aim of this study to is show the ability of (LAB) to produce antimicrobial activity as single or mixed cultures with/without different types of antibiotic to inhibit the growth of seven genera of pathogenic bacteria.

MATERIALS AND METHODS

Microorsanisms

1. Lactic acid bacteria (LAB)

Five strains of LAB were obtained from food sciences and biotechnology department, College of Agriculture, University of Basrah. These strains were identified as *Streptococcus thermophilus ; Lactococcus lactis* subsp. *lactis* ; *L. lactis* subsp *cremoris ; L. lactis* subsp. *diacetilactis ; Leuconostoc* sp.Using Polymerase Chain Reaction (PCR) (by Dr. Richard K. Robinson, Food Science end Technology Dept., University of Reading, UK). LAB were propagated twice in 10% skim milk at 37 C° for 16-18 hr. (13). The grown bacteria were cultured in MRS broth (Difco) at 40 – 45 C° for 18 – 24 hr.

2. Target bacteria

Seven genera of pathogenic bacteria (Marine bacteria Lab., Marine Environmental chemistry. Dept., Marine Science Center, University of Basrah) were isolated from different sources of water including (*Escherichia coli, Salmonella* sp., *Proteus* sp., *Klebseilla* sp., *Aeromonas* sp., *Staphylococcus* sp., and *Clostridium* sp) which were previously identified according to (6) has been tested for their resistance to antimicrobial activity and antibiotics.

Preparation of inoculum

Ten colonies of LAB and target bacteria which were grown on MRS agar and nutrient agar respectively at 37 C° for 24 hr, were transferred to test tubes containing 5 ml of nutrient broth and were incubated at 37 C° for 4 – 6 hr. The broth were diluted until the number of bacteria reached approximately 1x 10^7 ml^{-1} (2).

Determination of antimicrobial activity

1. Bacterial disk diffusion methods

The antimicrobial activity of LAB were tested as single or mixed culture (v/v). By spreading 0.1 ml of target bacterial broth on nutrient agar, dried for 15 min at room temperature . 6 to 8 holes were done with cork borer (7 mm in diameter). Using microsyringe 50µl of LAB culture were transferred to the holes and incubated at 37 C° for 18 – 24 hr. The diameter of inhibition zones were measured according to Baron and Finegold (2).

2.Bacterial supernatant

The lactococci broth were centrifuged at 2000 rpm for 10 min. The supernatant were separated and testing for their antimicrobial activity(2).

3. Determination of Minimum Inhibitory Concentration (MIC)

This method was used according to Baron and Finegold (2) to determined the minimum inhibitory concentration of LAB.

Statistical analysis

The results were analyzed using SPSS V.I 1 (14) program at level 0.1 and 0.05.

RESULTS AND DISCUSSION

Most of lactococci which were used as single or mixed cultures produced inhibition zones against target bacteria and this was agreed with Menash *et al.* (10), who found that lactococci inhibit some Gram positive or negative.

The antibacterial activity of (LAB) cells were much better than those of the supernatants, (Table 1), and this is may be to the presence of many antibacterial compounds in the wholes of (LAB) cells more than which found in their supernatants (13, 16).

The largest inhibition zone was in the synergistic action of *L. diacetilactis* and *Leuconostoc* sp. against most pathogenic bacteria except *Clostridium* which showed no effect by any single or mixed (LAB) cultures and this result agreed with (9), who reported that the resistance of the Gram - positive, spore forming bacteria to (LAB) perhaps due to the increase in formation of spores at acidic pH.

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Table 1. Antimicrobial activity of Lactococci cells and supernatant against target bacteria.

Lactococci type	Diameter of inhibition zone (mm)															
	Pro.		*E.coli*		*Cl.*		*Staph.*		*S.*		*A.*		*K.*			
	Bac.	*Sup.*	*Bac.*	*Sup.*	*Bac.*	*Sup.*	*Bac.*	*Sup.*	*Bac.*	*Sup.*	*Bac.*	*Sup.*	*Bac.*	*Sup.*	*Bac.*	*Sup.*
1	8	6	7	6	0	0	7	5	6	4	6	5	6	4		
2	9	7	9	6	0	0	7	5	8	5	9	7	7	7		
3	8	7	8	6	0	0	8	6	8	6	7	6	7	6		
4	6	6	7	7	0	0	6	6	6	6	7	7	7	7		
5	9	6	8	5	0	0	9	6	9	5	8	5	9	6		
1+2	11	9	15	13	0	0	15	11	9	7	12	10	11	9		
1+3	10	8	12	11	0	0	18	10	10	7	12	9	10	7		
1+4	7	6	6	5	0	0	6	5	5	5	5	5	5	4		
1+5	8	6	7	5	0	0	8	5	7	5	6	5	7	5		
2+3	10	8	10	7	0	0	9	7	10	7	11	8	9	8		
2+4	10	8	10	8	0	0	9	7	9	7	10	7	8	7		
2+5	13	10	15	9	0	0	16	12	11	9	14	10	13	9		
3+4	6	5	6	5	0	0	6	5	5	5	6	6	6	5		
3+5	10	8	12	8	0	0	14	12	11	9	13	10	12	8		
4+5	17	9	20	12	0	0	19	13	17	10	19	16	14	8		

1: *Streptococcus thermophilus* ; 2: *Lactococcus lactis* subsp. *lactis* ; 3: *Lactococcus lactis* subsp. *cremoris* ;4: *Lactococcus lactis* subsp. *diacetilactis* ;5: *Leuconostoc* sp.; Sup.: *Supernatant.* ; *E.coli*.: *Escherichia coli*.; *Cl*.: *Clostridium* sp. : *Staph*.: *Staphylococcu*: *S*.: *Salmonella* sp.; *A*.: *Aeromonas* sp.; *K*.: *Klebsiella* sp.

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In Table 2 the MIC values of the antibiotic cefotaxime was measured alone or mixed with single and mixed culture. The highest inhibiting effect was by the mixture of cefotaxime and *L. diacetilactis* and *Leuconostoc* followed by the mixture of cefotaxime and *L. lactis* and *Leuconostoc*.

The results of MIC values in Table 3 and 4 showed that *Clostridium* sp. (Gr^{+ve} bacteria) and *Klebseilla* sp.(Gr^{-ve} bacteria) were resistant to amoxicillin and clindamycin alone or mixed with LAB.

In Table 5 there were variations in MIC values for gentamycin towards pathogenic bacteria either alone or mixed with LAB cultures. *Proteus* spp. and *Staphylococcus* spp were more affected while *Clostridium* sp was less affected.

The mixture of ampiclox with single or mixed culture (Table 6) showed different inhibition activities against pathogenic bacteria.

In general the mixed cultures of *L. diacetilactis* with *Leuconostos* or *L. lactis* with *Leuconostoc* sp. were the most effective bacteria and this agreed with Branen *et al.*, (3), and Salminen *et al.* (12)who reported that some LAB exhibit antibacterial activity. This criteria may be related to chromosomal, transposons or plasmid related genes(5).

The most sensitive target bacteria were : *Staphylococcus* spp.(Gr^{+ve} bacteria) and *Proteus* spp.(Gr^{-ve} bacteria) , while the most resistant target bacteria were : *Clostridium* sp. (Gr^{+ve} bacteria) and *Klebseilla* sp. (Gr^{-ve} bacteria),the resistance for antibacterial agents in *Klebseilla* sp .was may be due to the presence of the capsule. These results were agreed with Ayad *et al.* (1).

Results of this study agreed with Timmerman (15) who reported that the combination of some LAB strains with certain antibiotics resulted in a wider antimicrobial spectrum as compared with antibiotics alone.

The statistical analysis showed significant difference between LAB cells and their supernatants against some pathogenic bacteria at level 0.01 and 0.05 with some exceptions.

***Table 2. The MIC of Cefotaxim and Lactococci against target* bacteria.**

Target bacteria		*Pro.*	*E.coli*	*Cl.*	*Staph.*	*S.*	*A.*	*K.*
Cefortaxim MIC (mg/ml)	Cef.	4	8	0	2	16	8	8
	Cef. + 1	2	8	0	2	8	8	8
	Cef. + 2	2	4	0	2	8	4	4
	Cef. + 3	4	4	0	2	16	4	8
	Cef. + 4	2	8	0	2	16	8	8
	Cef. + 5	2	4	0	2	8	8	8
	Cef. + (1+2)	4	4	0	4	8	8	8
	Cef. + (1+3)	2	8	0	2	16	4	4
	Cef.+(1+4)	4	8	0	4	16	4	8
	Cef. + (1+5)	2	4	0	2	8	4	4
	Cef. + (2+3)	4	4	0	2	8	8	4
	Cef. + (2+4)	2	8	0	4	16	8	8
	Cef. + (2+5)	2	2	32	2	8	4	4
	Cef. + (3+4)	4	8	0	2	16	8	8
	Cef. + (3+5)	2	4	0	4	16	4	4
	Cef. + (4+5)	2	2	16	2	4	2	4

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Table 3. The MIC of Amoxicillin and Lactococci against target bacteria.

Target bacteria		*Pro.*	*E. coli*	*Cl.*	*Staph.*	*S.*	*A.*	*K.*
Amoxicillin MIC (mg/ml)	Amx.	0	0	0	0	0	0	0
	Amx. + 1	32	32	0	16	32	16	0
	Amx. + 2	8	8	0	16	16	32	0
	Amx. + 3	32	8	0	16	32	32	0
	Amx. + 4	32	32	0	16	32	32	0
	Amx. + 5	16	8	0	16	32	32	0
	Amx. + (1+2)	32	16	0	8	32	32	0
	Amx. + (1+3)	16	32	0	16	32	16	0
	Amx. + (1+4)	32	32	0	8	32	32	0
	Amx. + (1+5)	16	8	0	16	32	32	0
	Amx. + (2+3)	32	16	0	16	32	16	0
	Amx. + (2+4)	16	32	0	16	32	32	0
	Amx. + (2+5)	8	8	0	16	32	32	0
	Amx. + (3+4)	16	16	0	16	32	32	0
	Amx. + (3+5)	16	16	0	16	32	32	0
	Amx. + (4+5)	8	8	0	8	32	16	0

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Table 4. The MIC of Clindamycin and Lactococci against target bacteria.

Target bacteria		*Pro.*	*E.coli*	*Cl.*	*Staph.*	*S.*	*A.*	*K.*
Clindamycin MIC (mg/ml)	Clin.	4	0	0	16	0	0	0
	Clin. + 1	2	32	0	16	0	32	0
	Clin. + 2	4	16	0	16	32	0	0
	Clin. + 3	4	32	0	8	0	32	0
	Clin. + 4	4	0	0	16	0	0	0
	Clin. + 5	4	32	0	16	32	0	0
	Clin. + (1+2)	2	32	0	32	32	32	0
	Clin.+(1+3)	2	0	0	16	0	16	32
	Clin.+(1+4)	4	0	0	32	0	8	0
	Clin.+(1+5)	4	32	0	16	32	8	32
	Clin. + (2+3)	4	32	0	16	16	16	0
	Clin. + (2+4)	4	0	0	32	32	16	0
	Clin. + (2+5)	2	32	0	32	8	8	32
	Clin. + (3+4)	4	0	32	16	32	32	0
	Clin. + (3+5)	4	32	0	32	16	16	0
	Clin. + (4+5)	2	16	0	4	8	8	16

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Table 5. The MIC of Gentamycin and Lactococci against target bacteria.

Target bacteria		*Pro.*	*E. coli*	*Cl.*	*Staph.*	*S.*	*A.*	*K:.*
Gentamycin MIC (mg/ml)	Gen.	2	16	0	2	16	8	0
	Gen. + 1	2	16	0	2	16	8	0
	Gen. + 2	2	16	0	2	16	4	0
	Gen. + 3	2	16	0	2	16	4	32
	Gen. + 4	2	8	0	2	8	8	0
	Gen. + 5	2	16	0	2	8	8	32
	Gen.+(1+2)	2	8	0	2	16	8	16
	Gen. + (1+3)	2	8	0	2	8	4	0
	Gen.+(1+4)	2	16	0	2	8	4	16
	Gen.+(1+5)	2	8	0	2	8	4	0
	Gen. + (2+3)	2	16	0	2	16	8	16
	Gen. + (2+4)	2	16	0	2	16	8	16
	Gen. + (2+5)	2	8	32	2	8	4	16
	Gen. + (3+4)	2	16	0	2	16	8	16
	Gen. + (3+5)	2	16	0	2	16	8	16
	Gen. + (4+5)	2	8	16	2	8	4	8

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Table 6. The MIC of Ampiclox and Lactococci against target bacteria.

Target bacteria		*Pro.*	*E.coli*	*Cl.*	*Staph.*	*S.*	*A.*	*K.*
Ampiclox MIC (mg/ml)	Amp.	2	8	0	2	4	2	16
	Amp. + 1	2	8	32	2	2	2	4
	Amp. + 2	2	4	32	2	4	2	16
	Amp. + 3	2	4	32	2	4	2	16
	Amp. + 4	2	8	0	2	4	2	8
	Amp. + 5	2	8	32	2	2	2	16
	Amp.+(1+2)	2	4	32	2	2	2	4
	Amp.+(1+3)	2	8	0	2	4	2	16
	Amp.+(1+4)	2	4	0	2	2	2	8
	Amp.+(1+5)	2	8	32	2	4	2	16
	Amp. + (2+3)	2	2	0	2	2	2	4
	Amp. + (2+4)	2	4	32	2	2	2	4
	Amp. + (2+5)	2	4	32	2	2	2	8
	Amp. + (3+4)	2	8	0	2	4	2	8
	Amp. + (3+5)	2	4	0	2	4	2	4
	Amp. + (4+5)	2	2	16	2	2	2	4

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