Antimicrobial activity of *Leuconostoc mesenteroides* biofilm against different microorganisms

Asmaa Sabah Ahmaed **Abdullah Ismaeel Awad**

Food Science / College of Agricultural Engineering Sciences / University of Baghdad
E-mail: asmsabah@yahoo.com , abdu20110@yahoo.com

Abstract

Back ground*: Leuconostoc* is one of the species of lactic acid bacteria that produced biofilms.Probiotic bacteria that produced biofilm has been used as naturopathy against different microbial pathogens.
Objective: This study was conducted to determine the antimicrobial activity of *Leuconostoc* biofilm , against 24 isolates (4 of 6 different types) of food borne pathogens including *Staphylococcus aureus* , *Salmonella spp, Escherichia coli* , *Klebsiella spp* , *Pseudomonas aeruginosa* , *Streptococcus mutans* , *Bacillus subtillus* , *Bacillus cereus, Bacillus sterothermophillus and Candida albicans.*
Materials and methods: using various concentration in vitro by filter paper disk diffusion method .
Result: The present study showed the potent antimicrobial activity of the *Leuconostoc mesenteroides* biofilm against the all tested bacterial pathogens except *Bacillus* species and yeast *Candida albicans*. Biofilm produced by *Leuconostoc mesenteroides* showed highest zone of inhibition (13mm) against *Escherichia coli* and lowest zone of inhibition (7.0mm) against *Streptococcus mutans* .
Conclusion: Consequently, *Leuconostoc mesenteroides* biofilm may be used as an antimicrobial agent in food products to prevent spoilage.

Keywords: Biofilm, Dextran, Exopolysaccharide, *Leuconostoc mesenteroides*, Antimicrobial activity.

Introduction

Biofilms are defined as microbial communities characterized by the cells that are attached to each other, embedded in a matrix of Extracellular Polymeric Sub-stances (EPS) (1).Lactic acid bacteria could produce bio-film. The most important part of the biofilm matrix, are extracellular polysaccharides (2). *Leuconostoc mesenteroides* biofilm matrix contains components dextran, proteins and nucleic acids (3), and had an active action against many microbes, fungus, viruses and parasites. It was found that this biofilm had more effect on gram negative bacteria such as *Escherichia coli* ,*Pseudomonas aeruginosa* , *Proteus mirabilis*, *Shigella flexneri* ,*Campylobacter jejuni, Salmonella typhimurium* and gram positive bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis* ,*Enterococcus faecalis* (1) .While the study of Hitendra (4) reported that Leuconostoc bacteria had antibacterial activity against *Xanthomonas* spp , *Erwinia* spp and some spoilage molds. Some *Leu. mesenteroides sp. mesenteroides* strains were found to have inhibitory activity against *Aeromonas hydrophila* , *Pseudomonas aeruginosa* and *Shewanella putrefaciens* (6). Many strains of *Leuconostoc mesenteroides* showed a high ability to inhibit *Listeria spp*. because they produce bacteriocin (leucocin B)(7). This study aimed to observe the effect of *Leuconostoc mesenteroides* biofilm on the growth of some bacteria and fungi that cause food spoilage.

Materials and methods

Biological material

In this study, *Leuconostoc mesenteroides* sub sp *mesenteroides* was isolated from Sauerkraut and then identified by using cultural, microscopical and biochemical tests according to (8).

Cultivation conditions

The cultivation of *Leuconostoc mesenteroides* bacteria is usually performed in MRS medium (9,10).

Production of biofilm

Biofilm production was achieved according to the standard mix of Awad and Ahmaed (11) method as follows : Modified MRS medium by addition 10% sucrose.

Preparation of different concentrations of biofilm

Different concentrations were prepared from *Leuconostoc mesenteroides* biofilm (1:2, 1:3 ,1:4) in addition of stock concentration , while the bacteria were poured in sterilized petridishes and maintained after hardening at 4 ^{o}C petridish considered to be as control sample (12).

- **Study of antibacterial activity for different concentrations of biofilm**

Stage 1

The test isolates bacteria were activated by transferring loopfull to the Nutrient broth from agar medium and incubated tubes in 37C for 18 hours (6) while yeast activated by transferring loopfull to PDA medium and incubated in 28C for 24 hours.

Stage 2

Filter paper disk diffusion Method was adopted by spreading 0.1 mL of test bacteria on Nutrient Agar (NA) while test yeast on PDA using L-shaped glass rode.4-6 sterilized paper disks (Whitman No.3) were prepared for each petridish at a diameter of 5mm and each disk placed on the culture medium was loaded with 10µL from different concentrations of *Leuconostoc mesenteroides* biofilm by micropipette(13) . All petridishes were incubated at 37 ^{o}C for 24 hrs for bacteria and 28° C for 48hr for fungi. Diameters of clear zones were calculated (14).

- **Study of antifungal activity for different concentrations of biofilm**

This activity was examined using Poisoned Food Technique(15) which relied on the radial growth of tasted molds by placing a piece of 0.5cm from a fungal culture at age of 5 days in the centre of cultural medium. Petridishes were incubated at 25 ^{o}C. The diameter of fungal culture of control treatment calculated as it reaches the edge of peridish. Colonies diameters were calculated. The inhibition percentage was determined as following equation:

% Inhibition= growth diameter rate of control sample - growth diameter rate of treatment sample*100

Results and discussion

In this study nine types of bacteria were used Five gram positive bacteria : *Staphylococcus aureus* , *Streptococcus mutans* ,*Bacillus subtilis* ,*Bacillus cereus*,*Bacillus stearothermophilus* and four gram negative bacteria : *Pseudomonas aeruginosa* ,*Salmonella spp*. ,*Escherichia coli* , *Klebsiella spp.* In addition one yeast (*Candida albicans)*. Results showed the inhibitory activity of *Leuconostoc mesenteroides* biofilm against *Staphylococcus aurues* by using different concentrations as the inhibition continued until 1:4 (Figure (1)and Table (1)) . Diameters of clear zones were (12,11,10,8) mm consequently.It also showed antibacterial activity of *E.coli* and *Pseudomonas aeruginosa* .The effectiveness of biofilm against *Staphylococcus aurues* may be due to metabolic products of bacteria *Leuconostoc mesenteroides* such as organic acids , diacetyl, CO2,hydrogen peroxide , and secondary metabolites (such as bacteriocin) (2). The gram negative bacteria (*E.coli*) were highly sensitive to the biofilm for all concentrations (13, 11,11,8) mm.While diameters of clear zones of growth of *Pseu. aeruginos* were (11mm,10mm,10mm and 9mm) consequently using different concentration of biofilm. *Leuconostoc mesenteroides* has highly inhibitory effect against *E.coli*ATCC25922 and *Pseu. aeruginosa*ATCC27853 and may be due to its production biosurfactant that has antimicrobial activity (16).It can also damage the cell membrane by interfering with phospholipids and membrane proteins (17). The results showed the ability of biofilm to inhibit the *Streptococcus mutans* in diameters of clear zones 7mm and 6mm of the first two concentrations.This is confirmed by Kang *et al*.,(18) that appear *Leuconostoc mesenteroides* can production exopolysaccharides especially soluble dextran (11). It discourages the formation of a biofilm matrix .Many *Leuconostoc mesenteroides* species produce many organic acids in addition to a group of antimicrobial compounds ,especially protein products called bacteriocines and like bacteriocines (such as Carnosin and Leuconocin) . This compound inhibited wild group of gram negative and positive bacteria (19; 2). Bacteriocins disrupts the cell wall system ,inhibits proteins or synthesizes nucleic acids.It can bind to cell wall components such as lipid molecules or surface sites sutible for binding, leading to direct cell degradation and thus reduced bacterial counts (20) .All *Bacillus spp*. was not affected by all biofilm concentrations because it counts as a spore former bacteria and this might due to its special mechanism that qualified these bacteria to resist the active materials existing in *Leuconostoc mesenteroides* biofilm .

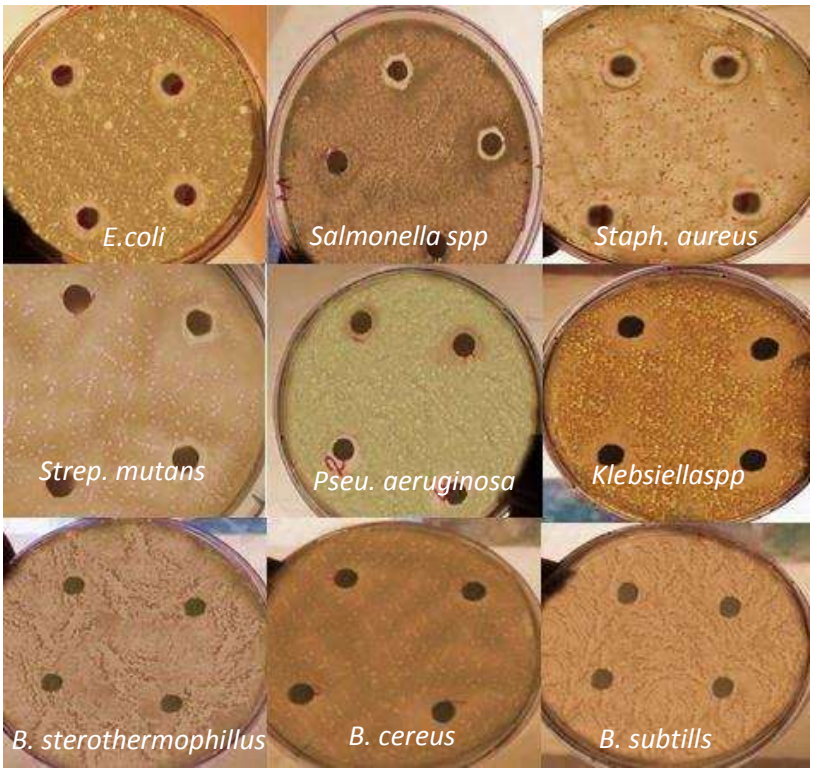

Figure (1): Inhibitory activity of *Leuconostoc mesenteroides* biofilm through clear zone

Table (1): Effect of *Leuconostoc mesenteroides* biofilm on some types of studied bacteria using disks paper method

Bacteria type	Growth inhibition clear zone average			
	1ml biofilm	1:2	1:3	1:4
Staph.aureus	12	11	10	8
Salmonella spp	10	9	NZ	NZ
E.coli	13	11	11	8
Klebsiella spp	10	10	9	8
Pseu.aeruginosa	11	10	10	9
Strep.mutans	7	6	NZ	NZ
B.subtilis	NZ	NZ	NZ	NZ
B.cereus	NZ	NZ	NZ	NZ
B.sterothermophillus	NZ	NZ	NZ	NZ
Candida albicans	NZ	NZ	NZ	NZ

***Inhibition disk is not involved the diameters of clear zones**
NZ=No Zone

Results of Figure (2) showed that the inhibitory activity percentage of *Leuconostoc mesenteroides* biofilm against *Penicillium spp.* mold as it reached 70.21%. That was higher than against *Aspergilus niger* mold as it reached 55.33% that what confirmed Hitendra *et al* .,(19) who observed that metabolic products for *Leuconostoc mesenteroides* such as bacteriocins Antifungal activity was attributed to many metabolitic products such as organic acids (that reduce pH) , H_2O_2 , CO_2 , diacetyle and bacteriocins(21).

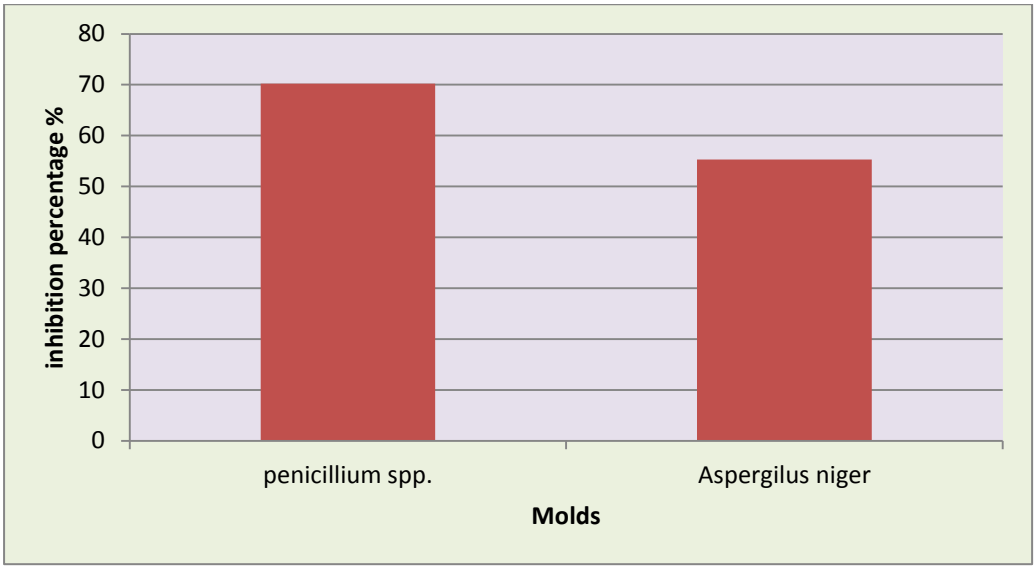

Figure (2): Effect of *Leuconostoc mesenteroides* biofilm on inhibition of some molds

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