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Probiotic Applications of Leuconostoc Mesenteroides: Antibacterial Activity Against MDR Pathogenic Bacteria

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Abstract. Leuconostoc mesenteriodes is an important probiotic bacteria with impact characteristics and benefits for human health. Last year, probiotic bacteria were used as an alternative to traditional antibiotics in treat of different diseases especially in the broad distribution of MDR bacteria. L. mesenteriodes isolate was purchased from (Al-Amin Center for Research and Biotechnology\ Najaf, Iraq) and re-diagnosis using MRS media in anaerobic condition then diagnosis by vitek 2 system. The antibacterial activity of L. mesenteriodes was assessed using the agar well diffusion method against MDR pathogenic bacteria isolated from different clinical cases. The L. mesenteriodes crude was obtained by centrifuging the bacterial isolates. Five different concentrations of probiotic bacteria crude were tested against MDR pathogenic bacteria revealing the antibacterial activity of L. mesenteriodes crude concentrations (stock, 90%, 75%, 50% and 25%) against MDR Staphylococcus aureus (22, 20, 20, 17, 14 mm), MDR Micrococcu. leuts (24, 23, 18, 16, 11 mm), MDR Proteus. mirabillis (22, 22, 18, 17, 14 mm), MDR Escherichia. coli (22, 20, 18, 12, 11 mm) and MDR Klebsiella pneumoniae were (20, 18, 14, 12, 11mm) an respectively. L. mesenteriodes probiotics improve antibacterial activity especially when used against multi-drug resistant bacteria

Highlights:

1. L. mesenteroides shows antibacterial potential against MDR pathogens.
2. Tested using agar well diffusion with five crude concentrations.
3. Effective against MDR bacteria strains including S. aureus, E. coli, and K. pneumoniae.

Keywords: probiotic, L. mesenteriodes, MDR, Crud extract, pathogenic bacteria.

Introduction

The emergence and widespread prevalence of multidrug-resistant (MDR) bacteria have posed significant challenges to modern medicine. Microorganisms were developed resistant against antibiotic due to the continuous exposure of these antibiotics (1). The expansion of MDR phenomenon rise the mortality and morbidity rates, for this causes they are named as 'Superbugs'. MDR is a natural process among microorganisms but the increasing of this phenomenon is attributed to many reasons such as the use of undefined antibiotics , bad health care services and unhygienic conditions (2).

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Conventional antibiotics are often ineffective against these pathogens, necessitating alternative treatment approaches. Probiotics are non-pathogenic and non-invasive microorganisms that have an important role in the enhancement of human health, when administered in suitable amounts (3). It have gained attention for their ability to suppress pathogenic bacteria. Among them, Leuconostoc mesenteroides (L. mesenteroides), a lactic acid bacterium, is noted for its beneficial health effects and antimicrobial properties (4).

This study aims to assess the antibacterial activity of L. mesenteroides against MDR bacteria, emphasizing its potential as a natural alternative to conventional antibiotics

Methods

Probiotic isolate and Culture Conditions

An isolate of probiotic L. mesenteroides was obtained from the Al-Amin Center and re-identified using de Man, Rogosa, and Sharpe (MRS) media under anaerobic conditions using polycarbonate Jar (TEMMEDIA/ India) then staining with gram stain and tested with biochemical test. Further confirmation of the isolate was performed using the VITEK 2 system.

Pathogenic bacterial isolates:

The pathogenic bacterial isolates were isolated from different clinical caseses then culture on (nutrient and Maconkey agar) and confirmed the diagnosis using vitek 2 compact system (biomeriux / France).

The antibiotic sensitivity test to each pathogenic bacterial isolate was determined using vitek AST compact system (biomeriux / France) to many antibiotics (Cefoxitin, Benzylpencillin, Oxacillin, Gentamicin, Tobramycin, Levofloxacin, Moxifloxacim, Erythromycin, Clindamycin, Linezolid, Teicoplanin, Vancomycin, Tetracycline, Tigecycline, Nittrofurantion, Fusidic Acid, rifampicin, Trimethoprim/sulfamethoxazole)

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Preparation of Crude Extracts

A portion of 1ml of L. mesenteroides isolate was used to inoculate 100 ml of MRS broth, in triplicate, and incubated anaerobically at 30ºC for the 96 hrs. Each culture was then centrifuged at 4 ºC, 10.000 rpm. for 20 min, after centrifugation of broth, cell-free supernatant was taken to complete antibacterial analysis (5).

The crude extract was diluent to 5 different concentrations. each concentration was obtained by diluent the crude extract with volume of sterile distal water (Table 1).

Table 1: Probiotic crude extract concentrations

Con. Percentage	**Crude extract + D.W.**
Stock (100%)	No dilution
90%	9ml+ 1ml
75%	7.5 ml+ 2,5 ml
50%	5ml+5ml
25%	2.5ml+7.5ml

Antibacterial Activity Assessment

The agar well diffusion method was employed to evaluate the antibacterial activity of 5 different concentrations of L. mesenteroides crude extracts against 5 isolates of MDR S. aureus, M. luteus, E. coli, K. pneumonia and P. mirablis. Zones of inhibition were measured to determine antibacterial efficacy (6).

Statistical analysis:

The results are presented as the mean of three or four replicates ± standard error (SE). Statistical analyses were performed using SPSS software (version 22). The data obtained were statistically analyzed to determine the degree of significance using one-way analysis of variance (ANOVA) at a probability level of $P \leq 0.05$ levels of statistical significance

Result and Discussion

The probiotic L. mesenteroides isolate was seem to be on MRS agar (Fig. 1). The figure showed the colonies of bacteria were small, round, and lenticular with a whitish colour.

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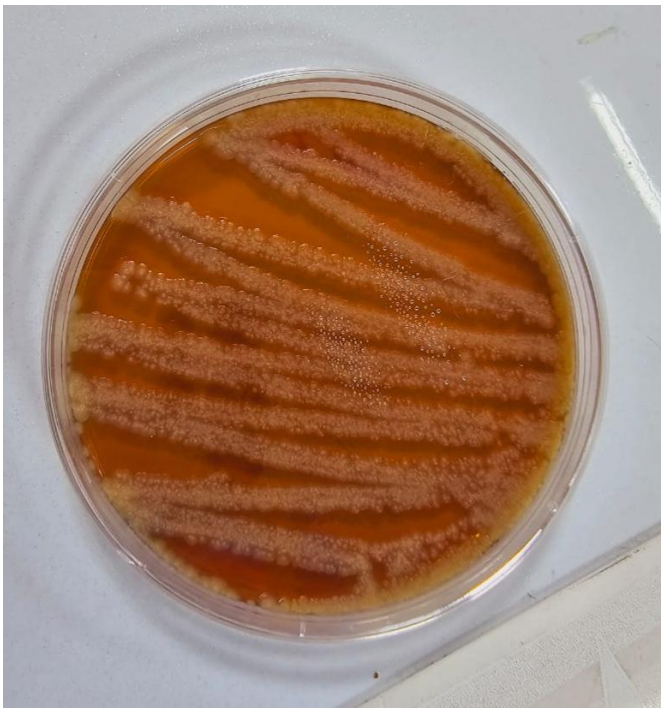

Figure 1: L. mesenteroides probiotic bacteria on MRS agar enrichment for 48hrs

According to re-identified the probiotic isolate, the microscopic appearance carried out after Gram staining revealed that L. mesenteroides were Gram-positive, ovoid-shaped in pairs and in even, short, and curved chains. These results are similar to those (7, 8, 9). The biochemical test results agree with the criteria specific to the genus Leuconostoc, were same to those obtained by (10, 11).

MDR Pathogenic bacteria:

Five types of pathogenic bacteria (S. aureus, M. luteus, E. coli, K. pneumonia and P. mirablis) were isolated from different clinical cases. Antibiotic susceptibility test were done by vitek 2 system for these isolates and its considered MDR because it has resistance to ≥3 classes of antibiotics. The antibiotic resistance percentage of Gram positive and negative pathogenic bacteria were illustrated in table 2 and 3 .

Table 2: antibiotic resistance percentage of pathogenic Gram positive bacteria

Cefoxitin	100% R	**50% R**
Benzylpencillin	100% R	**50% R**
Oxacillin	100% R	**50% R**

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Gentamicin	80% R	**25% R**
Tobramycin	75% R	**50% R**
Levofloxacin	50% R	**50% R**
Moxifloxacim	100% R	**40% R**
Erythromycin	100% R	**50% R**
Clindamycin	25% R	**25% R**
Linezolid	25% R	**25% R**
Teicoplanin	25% R	**25% R**
Vancomycin	25% R	**25% R**
Tetracycline	100%R	**50%R**
Tigecycline	25% R	**0% R**
Nittrofurantion	25% R	**5% R**
Fusidic Acid	75% R	**0% R**
Rifampicin	25% R	**20% R**
Trimethoprim/sulfamethoxazole	25% R	**5% R**

Table 3: Antibiotic resistance percentage of pathogenic Gram negative bacteria

Ticarcillin	100% R	100% R	**100% R**
Ticarcilli/clavulanic acid	50%R	100% R	**100% R**
Piperacillin	100% R	100% R	**100% R**
Piperacillin/Tazobactam	25%R	25%R	**100% R**
Ceftazidime	5%R	5%R	**20%R**
Cefepime	0% R	5%R	**5%R**
Aztreonam	5%R	5%R	**5%R**
Imipenem	0% R	25%R	**0% R**
Meropenem	5% R	0%R	**0%**
Amikacin	50% R	50% R	**50% R**
Gentamicin	25% R	50% R	**25% R**
Tobramycin	50% R	50% R	**25% R**
Ciprofloxacin	50% R	50% R	**100% R**
Minocycline	50% R	100% R	**50% R**
Colistin	25% R	25%R	**0% R**
Trimethoprim/Sulfamethoxazole	50% R	75% R	**80%R**

In this study, probiotic bacteria (L. mesenteroides) crude was screened in vitro for antibacterial against MDR pathogenic bacteria (S. aureus, M. leuts, E. coli, K. pneumonia and P. mirablis)

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The antibacterial activity of L. mesenteroides crude extracts against MDR S. aureus, M. leuts, E. coli, K. pneumonia and P. mirablis (Fig. 2).

The results indicate that L. mesenteroides crude extracts demonstrated a concentration-dependent inhibitory effect against all pathogens, with higher concentrations showing more significant inhibition zones. The statistical analysis revealed a significant differences of antibacterial activity of L. mesenteroides among MDR pathogenic bacteria at p value ≤ 0.05.

Although the pathogenic bacteria were MDR which resist more than three antibiotics and are difficult to treat, the antibacterial compounds present in probiotic bacteria (L. mesenteroides) had strong activity against bacterial growth.

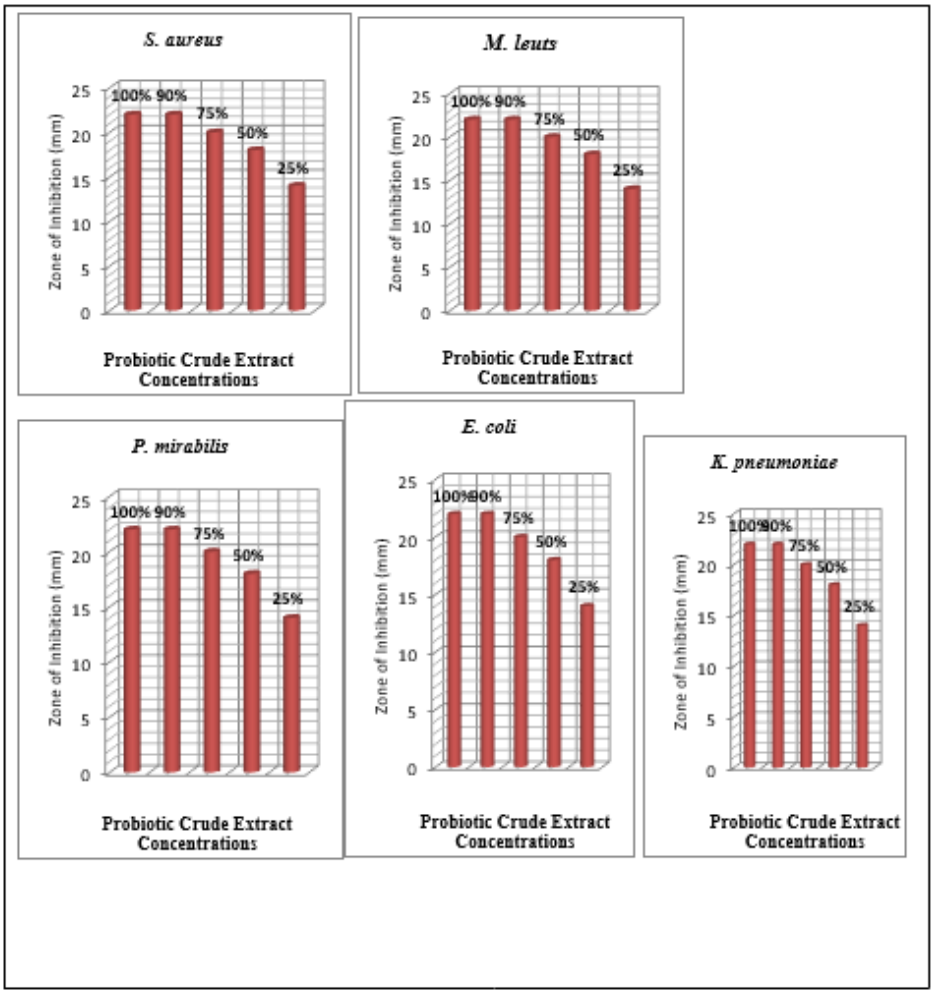

Figure 2: Inhibition zone (mm) of MDR pathogenic bacteria (S. aureus, M. leuts, E. coli, K. pneumonia and P. mirablis) as a result of L. mesenteroides crude extracts antibacterial activity.

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Discussion

The findings of this study demonstrate that L. mesenteroides exhibits strong antibacterial activity against MDR S. aureus, M. leuts, E. coli, K. pneumonia and P. mirablis. Certainly, probiotic bacteria metabolites have a vital role as antibacterial, antifungal and antibiofilm (12). In general the probiotic bacteria have the antibacterial activity properties and in especially the L. mesenteroides ability to produce antimicrobial compounds, such as organic acids and bacteriocins, likely contributes to its efficacy (13, 14). This highlights the potential of L. mesenteroides as an adjunct or alternative to traditional antibiotics, particularly in combating MDR pathogens.

Leuconostoc mesenteroides produces heat-stable bacteriocins. Bacteriocins are antibacterial peptides produced during fermentation, showing activity against closely related bacterial species (15).

Acidic conditions inhibit the growth of many pathogenic bacteria, as most pathogens do not thrive in low-pH environments. Lactic acid disrupts cell membrane integrity and inhibits metabolic enzyme activity in pathogens. Lactic acid was more effective than strong acids like hydrochloric acid due to its ability to permeabilize and disrupt bacterial membranes uniquely (16, 17). L. mesenteroides causes cell membrane disruption, cytoplasmic leakage, and cell lysis (18).

Many studies pointed L. mesenteroides showed potential as a probiotic alternative to combat antibiotic resistance (19, 20, 21). Probiotic bacteria demonstrate potent antibacterial and antibiofilm activities against uropathogenic E. coli (UPEC) at both genotypic and phenotypic levels (22). While other study (24) investigate the antibacterial activity of L. mesenteroides against pathogenic K. pneumoniae targeting the cell wall of the bacteria. L. mesenteroides crude extract exhibited antimicrobial activity against MDR S. aureus, E. coli, P. mirabilis and listeria monocytogenes in consistence with our study (25).

Probiotics like L. mesenteroides, L. acidophilus, and L. plantarum offer sustainable alternatives to antibiotics in veterinary and human medicine, particularly for combating multidrug-resistant pathogens bacteria (23)..

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Conclusion

The study demonstrates the robust antibacterial potential of Leuconostoc mesenteroides, characterized by broad-spectrum activity against MDR pathogenic gram positive and negative bacteria. These properties make them promising candidates for use as natural preservatives and therapeutic agents. Its use could represent a natural, effective approach to addressing the global crisis of antibiotic resistance. Further studies are warranted to isolate specific bioactive compounds and explore clinical applications. Further in vivo studies to confirm the efficacy and safety of LABs in clinical and agricultural settings

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