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Exploring the Antibacterial Potency of Bacteriocins

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Abstract: ***Background: Increasing antibiotic resistance has intensified the search for alternative antimicrobial agents. Bacteriocins are ribosomally synthesized antimicrobial peptides that can exhibit targeted inhibitory activity against bacteria. Objective: To assess and compare the in vitro antibacterial activity of bacteriocin extracts obtained from four Lactobacillus isolates, designated IS1, IS2, IS4, and IS6, against Salmonella typhi, Bacillus subtilis, and Streptococcus mutans. Methods: Extracts from the four isolates were evaluated using broth microdilution and microbial growth inhibition assays. Optical density measurements and inhibitory activity were assessed using a ProMax5 ELISA microplate reader (USA). Minimum inhibitory concentration (MIC) and half-maximal inhibitory concentration (IC50) values were compiled for each isolate–test organism combination. Results and conclusion: The Lactobacillus-derived extracts exhibited isolate-dependent activity against the tested Gram-positive and Gram-negative organisms. Numerical MIC and IC50 values were reported for all four isolates against B. subtilis and S. mutans, whereas the values for IS1 against S. typhi were recorded as NA. The findings support further investigation of these extracts as potential antibacterial agents; confirmation of the reported concentration values and additional characterization are necessary before therapeutic applications can be established.***

Keywords: bacteriocin; antibacterial activity; isolates; minimum inhibitory concentration (MIC); half-maximal inhibitory concentration (IC50).

I. INTRODUCTION

Antibiotic resistance is a growing global concern that has encouraged investigation of alternative antimicrobial agents. Bacteriocins are ribosomally synthesized peptides produced by bacteria and are recognized for their ability to inhibit susceptible bacterial strains, often including closely related organisms. Their antibacterial properties provide a basis for exploring targeted approaches to bacterial growth inhibition (Riley & Wertz, 2002).

A. Antibiotic resistance and the need for alternatives

The declining effectiveness of conventional antibiotics presents a substantial public health challenge and creates a need for additional antimicrobial strategies. Bacteriocins have attracted interest because their antimicrobial properties may complement existing approaches and help address some limitations associated with conventional antibiotics (Drider et al., 2006).

B. Bacteriocins as potential antimicrobial agents

As ribosomally synthesized peptides, bacteriocins possess characteristics that warrant investigation for antibacterial applications (Parvez et al., 2006). Their capacity to target susceptible bacteria offers a rationale for evaluating them as potential alternatives or complements to broad-spectrum antibiotics (Cotter et al., 2013). The present study examines the activity of extracts obtained from Lactobacillus isolates against S. typhi, B. subtilis, and S. mutans.

C. Isolation and characterization of the study isolates

The bacteriocin-producing Lactobacillus isolates were designated Isolate 1 (IS1), Isolate 2 (IS2), Isolate 4 (IS4), and Isolate 6 (IS6). They were selected on the basis of their potential antibacterial properties. In vitro assays and microbial growth inhibition studies were used to examine the interactions between the corresponding extracts and the selected test organisms (Corsetti & Settanni, 2007).

D. Experimental approach

The experimental approach involved testing the extracts and measuring bacterial growth using a ProMax5 ELISA microplate reader (USA).

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These measurements were intended to characterize inhibitory activity against the selected organisms (Hammami et al., 2009). The broader research aims also included understanding the mechanisms underlying inhibition and comparing bacteriocin potency with conventional antibiotics; however, mechanistic experiments and comparative antibiotic results are not reported in the supplied dataset.

E. Implications for antibacterial strategies

Understanding interactions between bacteriocins and susceptible bacteria may inform the development of targeted antibacterial strategies (Tagg et al., 1976). The present findings are intended to contribute to research on alternative antimicrobial approaches. Further characterization is needed to determine how bacteriocins could support efforts to address antibiotic resistance (Hammes & Hertel, 2009).

II. MATERIALS AND METHODS

A. Growth Media Reagents and Assay Conditions

De Man, Rogosa, and Sharpe (MRS) broth, nutrient broth, and Mueller–Hinton agar (Hi-Media Chemicals, India) were used for bacteriocin production, bacteriocin assays, and antibiotic susceptibility testing, respectively. Urea broth was used for the urease assay. Analytical reagents were prepared in the laboratory from their constituent chemicals (Nissen-Meyer et al., 2009). Chemicals used for the experiments and analyses were purchased from Hi-Media and Merck, India.

B. Broth Microdilution Assay

Broth microdilution testing was performed with reference to Clinical and Laboratory Standards Institute (CLSI) guidance. Stock solutions of the standard and bacteriocin preparations were prepared at 50 mg/mL. Twofold serial dilutions were used for antimicrobial testing at the following concentrations: 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039, and 0.020 mg/mL.

C. Measurement and Presentation of Inhibitory Activity

The ProMax5 microplate reader was used to obtain measurements for the microdilution assay. The resulting MIC and IC50 values were compiled separately for S. typhi, B. subtilis, and S. mutans. The supplied manuscript does not specify the inoculum density, assay wavelength, incubation duration, number of replicates, identity of the standard, or calculation procedures used to derive the reported endpoints.

III. RESULTS

The microdilution results obtained using the ProMax5 reader are presented in Tables 1–3. All numerical entries and NA entries are retained as supplied. Although the dilution concentrations are described in mg/mL in the methods, units are not explicitly stated in the original table headings and require confirmation.

A. Activity against Salmonella typhi

Table 1. MIC and IC50 values of bacteriocin extracts against Salmonella typhi

S.N.	Bacteria	MIC	IC50
1	Isolates 1 (IS1)	NA	NA
2	Isolates 2 (IS2)	0.161	0.101
3	Isolates 4 (IS4)	0.05	0.047
4	Isolates 6 (IS6)	0.055	0.039

IS4 and IS6 had reported MIC values of 0.05 and 0.055, respectively, compared with 0.161 for IS2. Their corresponding IC50 values were 0.047, 0.039, and 0.101. Both endpoints for IS1 were recorded as NA.

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B. Activity against Bacillus subtilis

Table 2. MIC and IC50 values of bacteriocin extracts against Bacillus subtilis

S.N.	Bacteria	MIC	IC50
1	Isolates 1 (IS1)	0.109	0.45
2	Isolates 2 (IS2)	0.15	0.148
3	Isolates 4 (IS4)	0.142	0.140
4	Isolates 6 (IS6)	0.142	0.118

MIC values ranged from 0.109 for IS1 to 0.15 for IS2; IS4 and IS6 each had a reported MIC of 0.142. IC50 values were 0.45, 0.148, 0.140, and 0.118 for IS1, IS2, IS4, and IS6, respectively.

C. Activity against Streptococcus mutans

Table 3. MIC and IC50 values of bacteriocin extracts against Streptococcus mutans

S.N.	Bacteria	MIC	IC50
1	Isolates 1 (IS1)	0.52	0.058
2	Isolates 2 (IS2)	0.193	0.185
3	Isolates 4 (IS4)	0.128	0.125
4	Isolates 6 (IS6)	0.132	0.130

IS1 had the highest reported MIC (0.52), followed by IS2 (0.193), IS6 (0.132), and IS4 (0.128). The corresponding IC50 values were 0.058, 0.185, 0.130, and 0.125, respectively.

IV. DISCUSSION

The MIC and IC50 results indicate variation in the inhibitory activity of the tested bacteriocin extracts across the three organisms. Interpretation depends on both the producing isolate and the test organism.

The results in Table 1 indicate that IS4 and IS6 had lower reported MIC values (0.05 and 0.055, respectively) than IS2 (0.161) against *S. typhi*. Their IC50 values followed a similar pattern: 0.047 for IS4 and 0.039 for IS6, compared with 0.101 for IS2. Thus, among isolates with numerical results, IS6 had the lowest IC50, whereas IS4 had the lowest MIC. These findings suggest differences in inhibitory activity among the extracts.

Numerical inhibitory endpoints were reported for all four isolates against *B. subtilis*. MIC values ranged from 0.109 to 0.15, with IS1 showing the lowest reported MIC, IS2 the highest, and IS4 and IS6 intermediate values of 0.142. The MIC results therefore suggest a relatively narrow range of inhibitory concentrations rather than identical activity. The IC50 values ranged from 0.118 to 0.45. In particular, the IS1 IC50 of 0.45 exceeded its MIC of 0.109. Bacteriocin research provides a context for investigating these differences (Drider et al., 2016).

Against *S. mutans*, MIC values indicated the following order from lowest to highest: IS4 (0.128), IS6 (0.132), IS2 (0.193), and IS1 (0.52). At the MIC endpoint, IS4 and IS6 therefore showed greater apparent potency than IS1. In contrast, IC50 values were lowest for IS1 (0.058), followed by IS4 (0.125), IS6 (0.130), and IS2 (0.185). The MIC and IC50 rankings consequently differed.

Overall, the reported results suggest that antibacterial activity depends on both the *Lactobacillus* isolate and the test organism. Numerical endpoints were recorded for three isolates against *S. typhi* and for all four isolates against *B. subtilis* and *S. mutans* (Cotter et al., 2005; Das & Nair, 2019).

V. CONCLUSION

The reported MIC and IC50 values suggest that extracts from Lactobacillus isolates IS1, IS2, IS4, and IS6 exhibit different inhibitory profiles against *S. typhi*, *B. subtilis*, and *S. mutans*. Responses against *S. typhi* varied among isolates. Numerical endpoints were reported for every isolate against *B. subtilis*, while the *S. mutans* results showed substantial variation in MIC values. These findings support consideration of isolate-specific responses when investigating bacteriocins as antimicrobial agents.

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Conflict of Interest

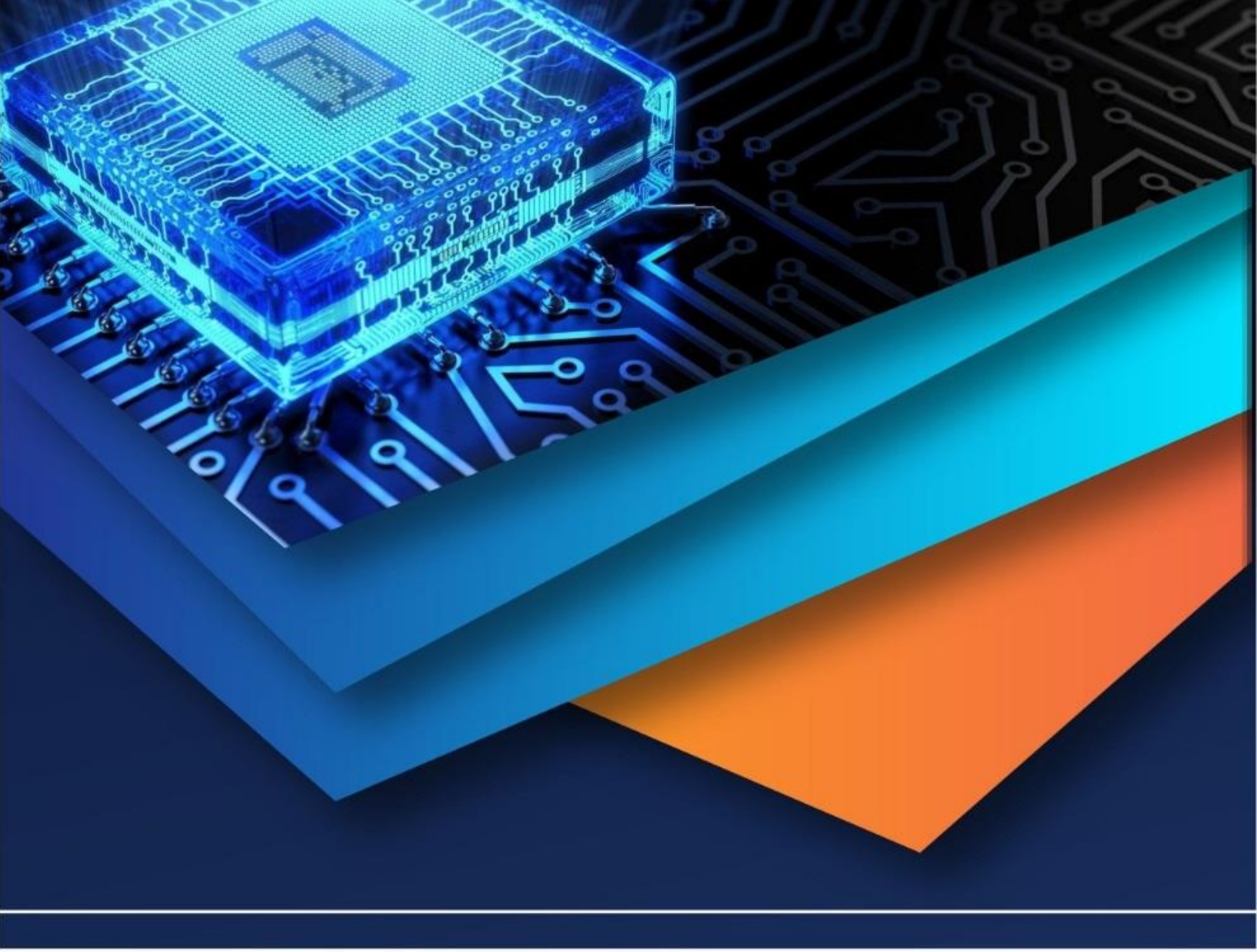
The authors declare no conflict of interest.

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