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An analysis of probiotic bacteria's ability to produce biological preservatives and the determination of their minimal inhibitory concentrations

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Abstract

Food safety is enhanced by the use of antimicrobials that prevent or delay spoilage and ensure widespread use of pesticides with drawbacks such as increased costs, concerns about food residues and toxins. This study evaluated the production capacity of antimicrobial proteins by two Enterococcus strains (Enterococcus faecalis and Enterococcus hirae). After the cultures of Enterococcus faecalis and Enterococcus hirae in BHI agar medium, the antimicrobial compounds of the strains were purified by dialyzing, and the amount of produced protein was determined by a Lowry test. Further, SDS-PAGE electrophoresis was used to estimate molecular weight. The well-diffusion method was used to test the antimicrobial properties of the compounds studied. Before and after dialysis, factors related to protein activity were determined. Additionally, compounds were evaluated for their minimum inhibitory concentrations. The results show that the most antimicrobial effect of antimicrobial species on the Bacillus cereus and more antimicrobial effects on the hirae species on Staphylococcus aureus.

Keywords: Enterococcus faecalis; Enterococcus hirae; antimicrobial proteins; biological preservatives.

Practical Application: The most antimicrobial effect of antimicrobial species on the Bacillus cereus and more antimicrobial effects on the hirae species on Staphylococcus aureus.

1 Introduction

Food preservation indicates the placement of microorganisms in an unfavorable environment to prevent their growth and shorten their survival or death (Fan et al., 2020). The possible response of microorganisms to such adverse conditions determines whether they will grow or die (Bouslah et al., 2017). The use of biological preservatives such as antimicrobials produced by microorganisms in food is an alternative way to control Listeria monocytogenes and other pathogens (Ayobami et al., 2020; Ferrari et al., 2021).

In recent years, consumers have become more concerned about the processed foods they buy and consume. Demand for natural, high-quality, preservative-free foods that are healthy and sustainable is a challenge for the food industry (Xiao et al., 2020; Abdel-Rahman et al., 2021). Public awareness of these dangers has increased the interest in finding healthier alternatives to synthetic chemical pesticides (Vozhehova et al., 2018). Infected with pathogenic microorganisms and the growing demand for healthy and high-quality food and the delay of the consumer headquarters from using chemical preservatives and synthetic antibiotics to inactivate or prevent the growth of spoilage and microorganisms (Zhang et al., 2021; Sabia et al., 2003). All pathogens led to research into the use of natural antimicrobials. Edible herbs, spices, and fragrant vegetables that have antimicrobial properties can be acceptable natural alternatives to chemicals (Toba et al., 1991). Essential oil is a volatile oily liquid that is obtained from various parts of the plant (tissue with plant seeds), comes, and is widely used as a flavoring in food (Teneva et al., 2021). Although their antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties have long been recognized, the recent tendency to use natural alternatives has led to new scientific data on these chemicals (Sullivan et al., 2020; Fraqueza et al., 2021). The antimicrobial properties of essential oils extracted from various plant species have been obtained using a variety of experimental methods (Maisnier-Patin et al., 1996). In order to separate plant essential oils in industry, several methods such as water distillation, steam distillation, extraction by solvent, and supercritical carbon dioxide have been reported in scientific sources (Özogul et al., 2022; Bollam et al., 2021). However, water distillation is the most common method of separating oil from aromatic and medicinal plants (Oliveira et al., 2021).

Lactic acid bacteria or their antimicrobial products have traditionally been used as natural food preservatives with extended shelf life and food safety (Aroutcheva-Alla et al., 2001). Biological preservatives refer to lactic acid bacteria or

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antibacterial products derived from them, such as acetic acid, lactic acid, hydrogen peroxide, antimicrobial enzymes, rutrin, bacteriocin, diacetyl, acetaldehyde, and acetone. Probiotic bacteria can act as microbial barriers against gastrointestinal pathogens by preventing pathogen binding, altering the immune system, and producing antibacterial compounds (Torres et al., 2020). The genus Enterococcus, as a probiotic bacterium, is able to ferment carbohydrates to lactic acid, and thus the gastrointestinal tract and inhibit the growth of pH bacteria reduce the pathogen (Park & Ha, 2020). These microorganisms increase the digestion of food and neutralize the enterotoxins of Escherichia coli by producing antitoxins (Cleveland et al., 2001). These bacteria can also produce various types of antibiotics and bacteriocins such as acidophyllin, acidoline, lactalin, nisin, and enterosin, thus killing many bacteria such as Staphylococcus, Salmonella, Shigella. The most important advantage of antimicrobial proteins derived from enterococci is that they are non-toxic, easy to digest, leave no residue in food, are resistant to acid and heat, and can increase health and longevity in many foods are used (Cunha et al., 2020; Hutapea et al., 2021).

2 Material and methods

The microbial culture media was BHI agar and BHI broth in this study. However, chemicals were Ammonium succinate, ammonium sulfate, hydrogen peroxide, glucose, galactose, mannitol, lactose, arginine, potassium hydrogen phosphate, sodium dihydrogen phosphate, serpentine reagent, copper sulfate, sodium bromide, sodium, and Acrylamide gel 18.5%. Two variants of Enterococcus strains (Enterococcus faecalis 1393 and Enterococcus hirae 1239) along with Gram-negative bacteria of Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Klebsiella Pneumonia was provided. The selection of these strain test strains was based on the gram-positive and gram-negative spectra of bacteria and cocci and their bacilli. Moreover, the molecular weight was estimated by SDS-PAGE electrophoresis (Jiang et al., 2020; Cen et al., 2020).

To prepare primer culture from Enterococcus strains, each of the powdered lyophilized bacterial strains was dissolved in 5 mL of BHI broth medium. Thus, a suspension of bacteria was created. Then, in order to grow the bacteria, the environment containing the bacteria was incubated at 37 °C for 48 hours.

After culturing these bacteria in plates containing BHI agar culture medium, the macroscopic characteristics of enterococcal colonies were examined in the depth of the culture medium, such as shape, consistency, color, size, and microscopic characteristics by hot staining.

2.1 Evaluation of bacterial growth curve based on optical density

Initially, several colonies of Enterococcus strains were added to the BHI broth medium (inoculation preparation), and a spectrophotometer read the sample's optical density. Bacteria were sampled from the medium at 0, 2, 4, 6, 8, 12, 16, 24, 48, and 72 hours. Then the growth curve of the bacteria was drawn.

2.2 Minimum inhibitory concentration

Dilution broth technique in liquid medium was used to determine minimum inhibitory concentration. For this purpose, 1.6, 1.2, 1, 0.8, 0.6, 0.5, 0.07, 0.6, 0.5, 0.03, 0.02, and 0.01 (g/L), dilutions of protein sediments with specific concentrations were prepared. Then, the 24-hour culture of each pathogenic bacterium was inoculated into tubes containing different dilutions of protein deposits.

Tubes containing different dilutions (depending on the amount of optical density) of pathogenic bacteria in comparison with tubes without protein deposits at intervals of 24, 48, and 72 hours of heating, samples were tested at 37 °C. Minimum inhibitory concentration was defined as the minimum concentration of growth-inhibiting protein deposits.

3 Results and discussion

3.1 Microscopic and macroscopic tests

As a result of microscopic tests, Enterococcus colonies as spherical purple colonies were observed under the microscope. However, in the macroscopic test, Enterococcus colonies on agar plates BHI were observed as the colony of tiny needles.

3.2 Evaluation of the antimicrobial activity of fermented extracts

The results of the diameter of the inhibition of the incomplete extract of the Enterococcus faecalis and Enterococcus hirae after 24 hours of heat at 37 °C are presented in Table 1.

The diameter of the two bacterial growth (Enterococcus faecalis and Enterococcus hirae) showed that the highest effect of antimicrobial species on the Bacillus cereus and more antimicrobial effects on the hirae species on Staphylococcus aureus.

3.3 Minimum bacteriostatic concentration

Determination of minimum bacteriostatic concentrations of antimicrobial proteins in faecalis and hirae species on four pathogenic bacteria are presented in Table 2.

According to the results of Table 2, the minimum bacteriostatic concentration of phenytoin on bacteria shows that the minimum bacteriostatic concentration of phenytoin on Klebsiella pneumoniae. The reason for the lowest level of minimum bacteriostatic concentration of antibiotic technical antibiotic than antimicrobial compounds is the power of antimicrobial effect of phenytoin than inhibit the growth.

3.4 Determining the production curve of antimicrobial compounds

The production curve of antimicrobial compounds over time in U/mL is shown in Figure 1.

According to the curves, the production of antimicrobial compounds in the first 4 hours of growth was almost zero; because of that, no metabolites were produced. However, with the growth of bacteria, these metabolites also increase gradually.

Table 1. Results of the diameter of the inhibition of the growth of enterococcus extracts after 24 hours of heating.

	Listeria monocytogenesis	Staphylococcus aureus	Bacillus cereus	Klebsiella pneumoniae
Enterococcus faecalis	27	29	28	12
Enterococcus hirae	32	34	30	15

Table 2. Minimum bacteriostatic concentration.

	Listeria monocytogenesis	Staphylococcus aureus	Bacillus cereus	Klebsiella pneumoniae
Enterococcus faecalis	1.1	0.85	1.0	1.9
Enterococcus hirae	1.7	0.7	1.7	2.1

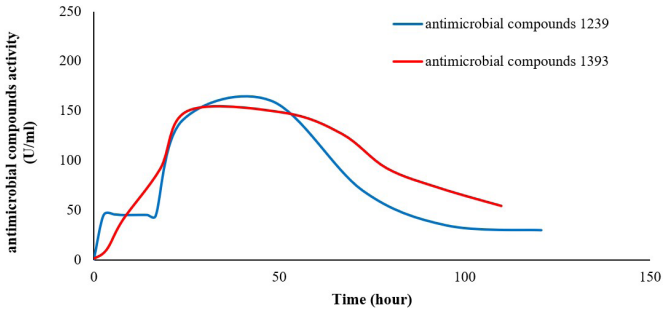

Figure 1. Production curve of antimicrobial compounds 1239 and 1393.

In the case of antimicrobial compounds 1393 and 1239, during 16 to 24 hours after initial inoculation, a sharp increase in production occurred. On the other hand, the production of these compounds has taken place in the logarithmic phase of growth. Therefore, it can be said that the production of antimicrobial compounds 1393 and 1239 follows the pattern of production of a primary metabolite. When the bacteria entered the dormant phase, the amount of these compounds produced remained almost constant and then gradually decreased. This reduction may be due to the action of protease enzymes released from dead cells. This finding is especially important in food when starter crops are used in the dairy industry because cellular proteases can rapidly break down these compounds.

4 Conclusion

Many lactic acid bacteria produce many different types of antimicrobial proteins. These compounds can be used as preservatives in many fermented and non-fermented foods. Currently, many antimicrobial proteins derived from enterococci, known as bacteriocins, are used as preservatives in food. It is necessary to extract bacteriocins produced from enterococcus, add them as natural starter cultures to food and, as a result, improve food safety and quality.

This study evaluated the production capacity of antimicrobial proteins by two Enterococcus strains (Enterococcus faecalis and Enterococcus hirae). Due to the maximum production of these compounds in the growth logarithmic phase, it was found that antimicrobial proteins produced by enterococcus are among the primary metabolites. Due to the production capacity of Enterococcus for the production of antimicrobial proteins, as biological preservatives, it can be used for long-term storage of food. Moreover, it can be reduced the use of chemical carcinogens significantly. Therefore, they significantly increase the shelf life of food, especially in dairy and meat products.

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