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ANTIMICROBIAL EFFECTS OF ORGANIC ACIDS AGAINST FISH PATHOGEN *YERSINIA RUCKERI*

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Abstract

Yersinia ruckeri is a common pathogen that causes mortality and economic losses in trout and salmon farming. The disease caused by this bacterium is also known as yersiniosis and enteric red mouth disease. This study aimed to investigate the antimicrobial effects of different organic acids such as sulfamic acid, fumaric acid, malic acid, lactic acid, itaconic acid, and tartaric acid against *Yersinia ruckeri* bacteria as alternatives to antibiotics. The antimicrobial effects of organic acids were determined by the disk diffusion method. As a result of the disk diffusion test, it was determined that all organic acids showed moderate inhibitory activity against *Yersinia ruckeri*. In addition, fumaric acid showed the highest antimicrobial activity with an 18 mm diameter. In addition, malic acid showed an inhibition zone of 16 mm, while tartaric acid and lactic acid both produced inhibition zones of 15 mm. Sulfamic acid and itaconic acid were found to be slightly less effective than the others, each producing an inhibition zone of 14 mm. In light of these findings, it can be said that the tested organic acids, especially fumaric acid, can potentially be used as an antimicrobial agent as an alternative to antibiotics to combat yersiniosis in aquaculture. In future studies, *in vivo* studies should be conducted by adding fumaric acid to fish diets and testing fish's resistance to yersiniosis disease.

Keywords: Fish disease, Yersiniosis, Organic Acids, Aquaculture, Alternative additives

Introduction

Today, the amount of animal aquaculture products obtained has reached 223.2 million tons (FAO, 2024). As is known, synthetic chemicals such as antibiotics and chemotherapeutics are used intensively in various stages of aquaculture to promote growth and combat diseases. However, excessive antibiotics and other drugs can harm fish health, animals, humans, and the aquatic environment. The use of antibiotics can cause fish pathogens to become resistant to antibiotics. For this reason, research on using organic acids in aquaculture has accelerated. Organic acids are an alternative to synthetic products due to their biological control agents and properties that increase fish health and nutritional value.

In 2024, the global market value of acidifiers most commonly used in the animal feed industry, including fumaric, lactic, and propionic acids, was estimated to be approximately USD 2.58 billion (Mordorintelligence, 2024). With the rapid increase in aquaculture production, the Asia-Pacific region dominated the feed acidifiers market in 2022, and fish feed alone accounted for 73.2% of the feed acidifiers market (Mordorintelligence, 2024). The ban on the use of growth-promoting antibiotics in animal feeds in Europe, and the tightening of regulations on the use of antibiotics as disease-preventive agents in the rest of the world are indicators that alternatives such as organic acids will be used more in the future (Ng and Koh 2017). Previous studies have reported that some organic acids, such as short-, medium-, and long-chain fatty acids inhibit the growth of various pathogenic bacteria (Ng and Koh 2017; Bolivar et al., 2018; Pereira et al., 2018; Jesus et al., 2019; Yamamoto et al., 2021). However, no study examines the antibacterial properties of fumaric, sulfamic, malic, tartaric, lactic, and itaconic acids on the fish pathogen *Yersinia ruckeri*. Therefore, this study aimed to investigate the *in vitro* antimicrobial effects of fumaric, sulfamic, malic, tartaric, lactic, and itaconic acids against the *Yersinia ruckeri*, which causes mortality and economic losses in rainbow trout, which is intensively farmed in the world. Thus, the aim was to shed light on *in vivo* studies with the findings obtained from this preliminary study.

Material and Method

Bacteria

The strain *Yersinia ruckeri* E42 (Genbank NO: KX388238) used in the study had been obtained from trout exhibiting signs of disease and was initially isolated by Dr. Ertan Emek ONUK.

Acid Materials

The fumaric, sulfamic, malic, tartaric, lactic acids and itaconic acid were obtained from the Alfasol® (Türkiye), and Acros Organics B.V.B.A. Belgium, respectively.

Antimicrobial Activity Tests

Disk diffusion test

Kirby-Bauer's disc diffusion method was used for the disk diffusion test (Bauer et al., 1966). For this purpose, the fish pathogen was grown in a Mueller-Hinton liquid culture medium at 22 °C incubation temperature. Afterward, the solid medium was transferred, the colonies showing the best growth were selected, and their density was adjusted to 0.5 McFarland in liquid medium. The density of the liquid medium is transferred to the Mueller-Hinton solid medium with a sterile cotton swab. After the transfer, 20 µl of fumaric, sulfamic, malic, tartaric, lactic, and itaconic acids (2 mg/disc) were dissolved in the appropriate solvent and impregnated with sterile disks for the disk diffusion test. The acid-impregnated sterile disks were placed on the solid medium, and the zones formed after 24 hours of incubation at 22 °C were measured. All studies were carried out in three replicates. Inhibition zones were measured following incubation, with zones smaller than 12 mm deemed ineffective, those ranging from 12 to 20

mm considered moderately effective, and zones exceeding 20 mm classified as highly effective (Rota et al., 2008).

Results

The disk diffusion test results of organic acids on *Yersinia ruckeri* shown in Figure 1. It was observed that tartaric acid produced a zone diameter of 15 mm, sulfamic acid 14 mm, fumaric acid 18 mm, malic acid 16 mm, lactic acid 15 mm, and itaconic acid 14 mm.

Figure 1. Results of disc diffusion testing of organic acids, 1: tartaric acid, 2: sulfamic acid, 3: fumaric acid, 4: malic acid, 5: lactic acid, 6: itaconic acid.

Discussion

The first disease record of *Yersinia ruckeri* in rainbow trout was reported in the late 1950s (Rucker, 1966). Today, it causes economic losses in all salmon and trout production areas globally (Chandra, 2024; Abdel-Latif et al., 2024).

This study focused on the effectiveness of organic acids such as sulfamic acid, fumaric acid, malic acid, lactic acid, itaconic acid, and tartaric acid in controlling *Y. ruckeri* as an alternative to antibiotics.

The results showed that all organic acids tested moderately inhibited *Y. ruckeri*. Similarly, Yamamoto et al. (2021) reported that butyrate and propionate organic acids had inhibitory effects against *Aeromonas hydrophila* and *Streptococcus agalactiae*. A different study revealed that butyrate, acetate, and propionate had higher inhibitory effects against *Vibrio alginolyticus*, while propionate had higher inhibitory effects against *Aeromonas hydrophila*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Bolivar et al., 2018). The study also examined the impact of combining organic acids with probiotic bacteria on pathogens and showed that fumarate was effective against *Streptococcus agalactiae* when combined with the probiotic *Lactobacillus plantarum* (Bolivar et al., 2018). In addition, Jesus et al. (2019) found that sodium butyrate effectively inhibited *Aeromonas hydrophila* and *Streptococcus agalactiae*. In addition, calcium and sodium propionate were reported to be quite effective in inhibiting *Aeromonas hydrophila* and *Citrobacter freundii* (Pereira et al., 2018).

In conclucion

This study found sulfamic acid, fumaric acid, malic acid, lactic acid, and itaconic acid to be moderately effective against *Yersinia ruckeri*. In future studies, in vivo studies should be conducted by adding fumaric acid to fish diets and testing fish's resistance to yersiniosis disease.

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Ethical approval

For this type of study, formal consent is not required.

Informed consent

Not available

Data availability statement

The authors declare that data are available from authors upon reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

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Contribution of authors

Abdullah Talha YAVILIOGLU: Formal analysis, Writing original draft

Sevdan YILMAZ: Project administration, Conceptualization, Data curation, Investigation, Methodology, Writing original draft

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