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Influence of Caffeic Acid on the Inhibition of *Yersinia ruckeri*

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Abstract

In this study, the effect of caffeic acid on growth and survival of *Yersinia ruckeri* was examined by exposing the pathogen to 0, 0.01, 0.05, 0.1, 0.5 and 1% concentrations of caffeic acid solution for 24 hours. The activity growth and survival of bacteria cells in exposed to each of 0.5 and 1% concentrations was found higher than that in controls and the differences were statistically significant ($p<0.05$). But, no differences were found in the survival of *Y. ruckeri* between the control and other experimental groups ($P>0.05$). The antibacterial effect of caffeic acid against *Y. ruckeri* was demonstrated. Further investigations are necessary to study the *in vivo* effect of the drug and validate the benefit of using caffeic acid in preventing infection in fish.

Keywords: Caffeic acid, *Yersinia ruckeri,* bacterial growth, survival

INTRODUCTION

Enteric Red Mouth (ERM) or Yersiniosis, caused by *Yersinia ruckeri*, is an acute/sub-acute disease of fish characterized with septicaemia and high mortality. ERM has become one of the most important bacterial diseases in rainbow trout breeding [1, 2]. Although rainbow trout at young ages are more susceptible to infection, all the wild and cultural salmonid fish may be affected. The economical losses attributed to ERM disease may be enormous unless early and accurate diagnosis and necessary treatment strategies are applied. The mortality can be as high as 70% in untreated farms. Approximately 60-70% of the infected fish may become carrier without showing any clinical signs of the disease [3].

Phenolic acids have received much attention because of their role in preventing bacterial infections due to their antibacterial properties. Hydroxy cinnamic acids are the major classes of phenolic compounds, which are found in almost every plant [4]. Hydroxy cinnamic acids such as caffeic acid (3,4-dihydroxy cinnamic acid) is one of the most common phenolic acids which occur in fruits, grains, and dietary supplements [5]. Caffeic acids (CA) are common representatives of a wide group of phenylpropane-derived compounds which are in the highest oxidation state. The caffeic acid has been shown to be effective against viruses, bacteria, and fungi [6]. It has been demonstrated that some polyphenols of the hydroxy cinnamate group, including caffeic acid are able to inhibit the growth of bacteria, including *E. coli, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes*, and some yeasts [7]. In addition, Caffeic acid has shown activity against the growth of *Legionella pneumophila* [8], other enterobacteria [9] and *Streptococcus mutans* [10, 11]. In the present study, the inhibitory effect of caffeic acid on *Y. ruckeri* was investigated.

MATERIALS and METHODS

Bacteria

Y. ruckeri strain, originally isolated from a rainbow trout with natural yersiniosis disease, was used in antibacterial susceptibility experiments. The isolate was grown in tryptic soy broth (TSB, Difco Laboratories) for 24 h at 25oC and then the culture isolate was frozen in 0.2 ml aliquots at -80oC. The isolate used in this study was prepared by inoculating 200 ml of TSB in a 500 ml culture flask with a thawed aliquot of the frozen isolate. Isolate of *Y. ruckeri* was then confirmed by standard biochemical tests as described in Bergey's Manual of Determinative Bacteriology [12] as well as API-20E biochemical analysis.

Experimental Plan

The bacterial strain was cultured overnight in TSB. To start the growth, 2 ml cultured *Y. ruckeri* stocks was added to 100 ml of TSB medium containing 0, 0.01, 0.05, 0.1, 0.5 and 1% CA (Sigma-Aldrich). The bacteria was grown for 24 h at 25°C. Optic density (OD) measurements at 600 nm were used to monitor the concentration of bacteria.

In trial II, for cell viability, the same bacterial strain also was grown on TSB with 0, 0.01, 0.05, 0.1, 0.5 and 1% of CA. For this experiment, different dilutions of *Y. ruckeri* bacteria (10^2 to 10^7) were prepared in TSB, and 20 µL of bacterial suspension was poured into control and experimental plates. The plates were incubated at 25°C for 24 h.

Data Analysis

The data experimental and control were compared with Student's t-test. All experiments were performed in three replicates and the values presented are the average of experiments with ±1 to ±5% standard deviation.

RESULTS

Fig. 1 shows the inhibitory effects of caffeic acid against *Y. ruckeri*. Increasing the concentration of caffeic acid in the optic density resulted in decreased bacterial growth. In the group of 0.01% caffeic acid, the optical density decreased 14%, while in the group of 1% caffeic acid, 39.80% ($p<0.05$) in comparison with the control group. The growth of Y.ruckeri was only moderate influenced by 0.01, 0.05 and 0.1% caffeic acid concentrations. But, at 0.5 or 1% concentrations of caffeic acid, growth of Y.ruckeri was decreased. We showed that the concentration and optical density of *Y. ruckeri* bacteria increased if caffeic acid concentration decreased and at 0.01%, caffeic acid in culture medium the optical density became similar to the control (Fig 1).

The significant differences were observed among the levels of 0.01 and 0.05% caffeic acid. However, a caffeic acid concentration of 0.5 and 1% or greater significantly decreased the number of bacterial colonies per plate compared with the control. In control plates showed 503 ± 61 bacteria colonies and in experimental plates with 0.5 and 1% of caffeic acid 88 ± 13 and 46 ± 24, respectively, bacteria colonies (Fig 2).

DISCUSSION

The results of this work demonstrate that caffeic acids has a antibacterial effect against *Y. ruckeri*. Caffeic acid reduced the numbers of Y.ruckeri cells, grown to the stationary phase. From this study, it can be concluded that phenolic acids such as caffeic acid may play an important role in the survival and growth of Y.ruckeri in broth.

Campos et al. [13], reported that caffeic acid caused membrane damage on a Gram-positive bacterium, *Oenococcus oeni*. Vaquero et al. [14] also reported recently that caffeic acid significantly affected growth of *Listeria monocytogenes*. But, antibacterial effect against *Y. ruckeri* of caffeic acid are still not known. The present study suggest that treatment of the bacteria with different doses of caffeic acid caused a dose-dependent decreased in bacterial viability. The results indicate the important role of caffeic acid in the reduction of *Y. ruckeri*.

The antibacterial activity is a potential mechanisms by which caffeic acid may contribute to reduce growth of bacteria. In fact, a direct relation between antibacterial effect and caffeic acid was shown in this study like the previously literature [10, 15]. In this study, inhibition of bacterial growth was observed on broth culture suplemented with caffeic acid. It is noteworthy that inhibition depends on the duration of caffeic acid exposure, as well as on the initial bacterial test level. Otherwise, caffeic acid in broth culture caused mainly a growth delay of *Y. ruckeri* (Fig 1). The growth of *Y. ruckeri* decreased as the time-dependent in broth culture.

Puupponen-Pimia et al. [16], showed that interaction of caffeic acid caused the antibacterial effect against *E. coli* and *Salmonella enterica*. In their study, low concentrations of caffeic acid (1 mg/L) were inhibitory to the growth of an *E. coli* strain isolated from human. Almajano et al. [17] demonstrated the antimicrobial activity of caffeic acid. The authors also showed that the minimum concentration of caffeic acid required to inhibit some microorganisms in the pH range of 5 to 7. They reported that a 0.4% (w/w) caffeic acid solution was sufficient to inhibit the growth of some of the studied microorganisms in the pH range of 5 to 7. The results in this study are in aggrement with the results in previous investigations. On the other hand, similar findings have also been reported by [7, 8, 9, 11, 15].

The antibacterial effect of caffeic acid against *Y. ruckeri* was demonstrated. It is anticipated that in the future, by determining the concentration of effective compounds, the antimicrobial activity of certain types of caffeic acid will possibly be predicted. Further *in vivo* studies are necessary in order to obtain evidence of benefits of caffeic acid on fish health.

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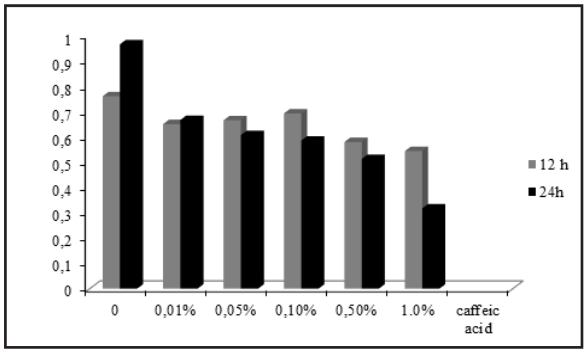

Figure 1. Growth of *Y. ruckeri* under diffrent caffeic acid doses.

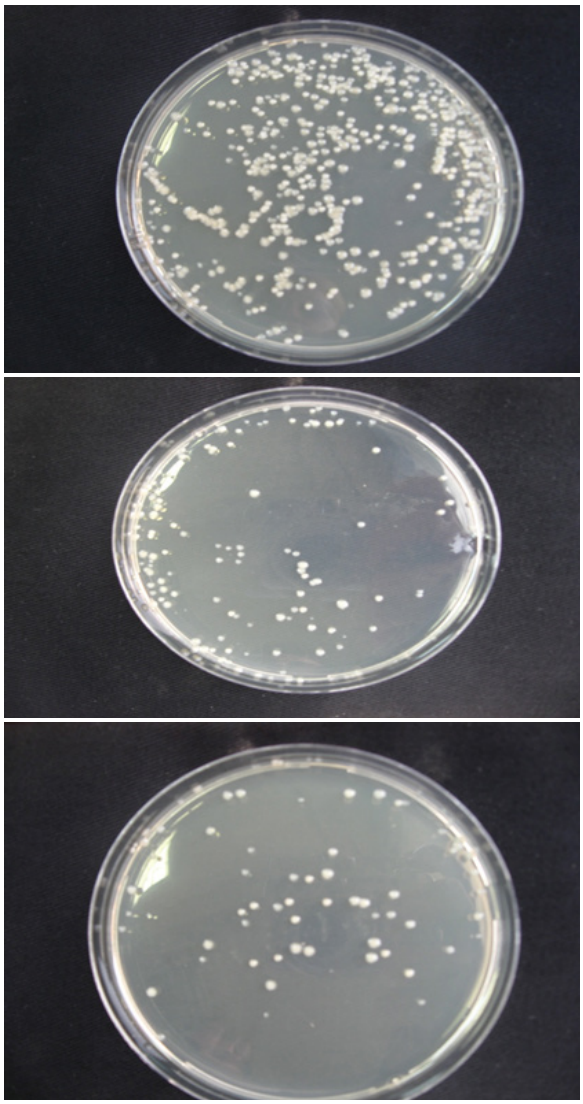

Figure 2. *Y. ruckeri* growing on TSB medium without caffeic acid (A), and with 0.5% of caffeic acid (B), and with 1% of caffeic acid (C)