

INACTIVATION EFFECT OF CHLORINE DIOXIDE ON PHYTOPATHOGENIC BACTERIA IN IRRIGATION WATER

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ABSTRACT

Chlorine dioxide (ClO₂) is a disinfectant which possesses high efficiency, low toxicity, fast, and broad sterilization ability. Although it can effectively disinfect many microorganisms including *Bacillus sp.*, *Staphylococcus sp.*, *Escherichia coli*, fungi and alga, the application of chlorine dioxide in irrigating water system of agriculture needs to be evaluated further. This study is, therefore, to investigate the germicidal efficacy of ClO₂ on pathogens of calla lily (*Erwinia carotovora* subsp. *carotovora* 3 and *E. carotovora* subsp. *carotovora* 7) and water convolvulus (*Ralstonia solanacearum* 3 and *R. Solanacearum* 7.) The results showed that ClO₂ at concentration of 1.3 and 13 mg L⁻¹ has significantly bactericidal efficiency against these pathogens. Moreover, the inhibition rate of ClO₂ solution (13 mg L⁻¹) was relatively constant at pH from 5 to 9.

INTRODUCTION

Chlorine dioxide (ClO₂) is a well-known disinfectant which possesses high efficiency, low toxicity, and broad sterilization ability against many harmful microorganisms in water. In 1950s, the use of ClO₂ was applied to disinfect the source water [1]. ClO₂ is also an effective disinfectant for the control of plant disease caused by plant causing bacteria including *Pythium spp.*, *Fusarium spp.*, *Colletotrichum spp.*, *Helminthosporium spp.*, *Streptomyces spp.*, *Alternaria spp.* and *Thielaviopsis spp.* [2,3]. The runoff and irrigation water return flow from containerized nursery and greenhouse facilities may contain nitrogen, various salts, plant pathogens and pesticides [4]. Thus, the

potential plant disease can be introduced through irrigation water. For instance, Pottorff and Panter demonstrated that *Pythium dissotocum* and *Pythium rostratum* were detected in irrigation water [5]. Until now, the use of chemical pesticides is still considered to be one of the main control methods for the plant diseases. Therefore, irrigation water is liable to be polluted by chemical pesticides. To conquer these problems, the development of an alternative disease control method is very important.

Calla lily (*Zantedeschia spp.*) is a popular floral crop being used as potted plants and cut flowers. Water convolvulus (*Ipomoea spp.*) is also an important water-cultivating vegetable in summer in Taiwan. Recently, *Erwinia carotovora* and *Ralstonia solanacea-*

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rum have been shown to cause the bacterial soft rot disease of calla lily and bacterial wilt of water convolvulus, respectively. Their infection rates were up to 51% in the cultivation region. They have apparently become major limiting factors for cultivation of calla lily and water convolvulus in Taiwan [6,7]. For this reason, the disinfection application of ClO₂ solution on phytopathogenic bacteria including *E. carotovora* subsp. *carotovora* 3 (Ecc 3), *E. carotovora* subsp. *carotovora* 7 (Ecc 7), *R. solanacearum* 3 (Rs 3), *R. solanacearum* 7 (Rs 7) provided from Department of Plant Pathogen, National Chung-Hsing University, in irrigation water were evaluated.

EXPERIMENTAL METHODS AND MATERIALS

1. Preparation of ClO₂ Solution

Chlorine dioxide solution was kindly provided by Zhu-Yi Biochemical Technology Co. Ltd., Taiwan, which was prepared by electrolytic generator. The concentration of ClO₂ was analyzed using the N,N-diethyl-p-phenylenediamine method [8] and used in the following tests.

2. Bacterial Strains and Culture Conditions

Ecc 3 and Ecc 7 were isolated from the soft rot disease of calla lily (*Zantedeschia spp.*) and Rs 3 and Rs 7 from bacterial wilt of water convolvulus. Subsequently, the tested bacteria were cultured at 37 °C in Luria-Bertani (LB) medium (pH 7.0) containing tryptone 1%, yeast extract 0.5% and sodium chloride 1%. When tested, the microorganisms were subcultured on the LB agar (1.5% w/v) medium for 18-24 h. The bacterial concentration was adjusted to the final concentration (ca. 10⁶-10⁷ cells mL⁻¹) according to the optical absorbance of bacteria at the wavelength of 600 nm.

3. Bactericidal Test

Microbial growth/inhibition can be determined in numerous ways such as direct microscopic counts, plate counts (viable counts), dry weight, turbidity measurement, absorbance, bioluminescence, among others [9,10]. However, when there are large amount of samples need to be measured in parallel, a simple, high-throughput, and quantitative method was necessary. tetrazolium salt (2,3,5-triphenyltetrazolium chloride, TTC) has been widely used as a growth indicator because of its high correlation between the growth of viable cells and absorbance [11-13]. Therefore, TTC was used as a growth indicator for evaluating the bactericidal efficiency of ClO₂ solution in this work [14]. Briefly, various concentrations (0.13, 1.3 and 13 mg L⁻¹) of ClO₂ solution diluted with sterilized irrigation water (pH 8.4; conductivity 5.47 mS cm⁻¹) collected

from field of Beidou county (central Taiwan) were mixed (9:1; v/v) with each tested bacteria at room temperature. For comparison, sterilized distilled water was used as control. After each time period, 0.5% Na₂S₂O₃ solution was added to the treatments to stop the reaction. Then 100 μL of each sample was pipetted into wells of a 24-well plate (CELLSTAR) containing 900 μL LB medium. 10 μL of stock TTC solution as a bacterial growth indicator were added to all wells and plates were incubated at 37 °C for 4 h. An acid-isopropanol (200 μL of 0.04 M HCl in isopropanol) solution was then added to all wells and mixed thoroughly to dissolve the violet crystals. The solutions were read with a spectrophotometer at 540 nm and the optical absorptions [A] were obtained. The inhibition rate (%) can be calculated according to $([A]_i - [A]_t)/[A]_i$, where [A]_i is the optical absorption of untreated bacteria.

Moreover, in order to further investigate the influence of ClO₂ solution under the environment of different pH values on plant pathogens, the different buffer solutions including acetate buffer (pH 5.0), phosphate buffer (pH 6.0, 7.0 and 8.0), and carbonate buffer (pH 9.0) were used to dilute the ClO₂ stock solution and assessed.

RESULTS AND DISCUSSION

1. Time Course of Inhibition Rate of ClO₂ Solution on Phytopathogenic Bacteria

As shown in Fig. 1, all the inhibition rates of ClO₂ solution (13 mg L⁻¹) on phytopathogenic bacteria in irrigation water at room temperature were approximately 94% after 5 min. However, all plant pathogenic bacteria were almost completely killed after 10

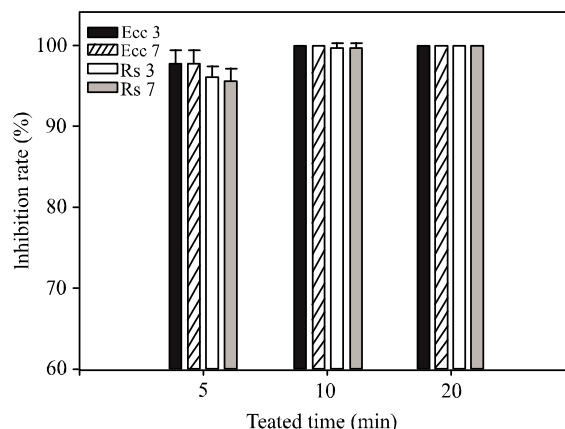


Fig. 1. Inhibition rate of ClO₂ solution (13 mg L⁻¹) on phytopathogenic bacteria treated with 5, 10 and 20 min. The initial bacterial concentration was approximately 10⁶-10⁷ cells mL⁻¹ according to the optical absorbance of bacteria at the wavelength of 600 nm. *E. carotovora* subsp. *carotovora* 3 (Ecc 3), *E. carotovora* subsp. *carotovora* 7 (Ecc 7), *R. solanacearum* 3 (Rs 3), *R. solanacearum* 7 (Rs 7). n = 3.

min. Therefore, all subsequent tests of bactericidal efficiency of ClO₂ solution were performed for 10 min.

2. Bactericidal Efficiency of Various Dosage of ClO₂

As shown in Fig. 2a, the results showed that the inhibition rates of 1.3 and 13 mg L⁻¹ against Ecc 3 and Ecc 7 placed in distilled waters were 100%. Meanwhile, the bactericidal efficiency of ClO₂ solution on Rs 3 and Rs 7 was also higher than 99% under the same treatment condition. However, the killing effects of ClO₂ solution on tested bacteria were apparently dropped as the concentration of disinfectant was reduced to 0.13 mg L⁻¹. We further examined the bactericidal efficiency of ClO₂ solution on phytopathogenic bacteria in irrigation water environment. Figure 2b shows similar patterns as in the case of distilled water, albeit the efficiency is lower at 0.13 mg L⁻¹.

3. Effect of pH Value on the Germicidal Efficiency of ClO₂

The results show that the effect of different pH value on the germicidal efficiency of ClO₂ has no significant difference (Fig. 3). As described previously, ClO₂ is a strong oxidizing agent and is able to disinfect effectively many microorganisms such as endospores, virus, fungi, and some common bacillus

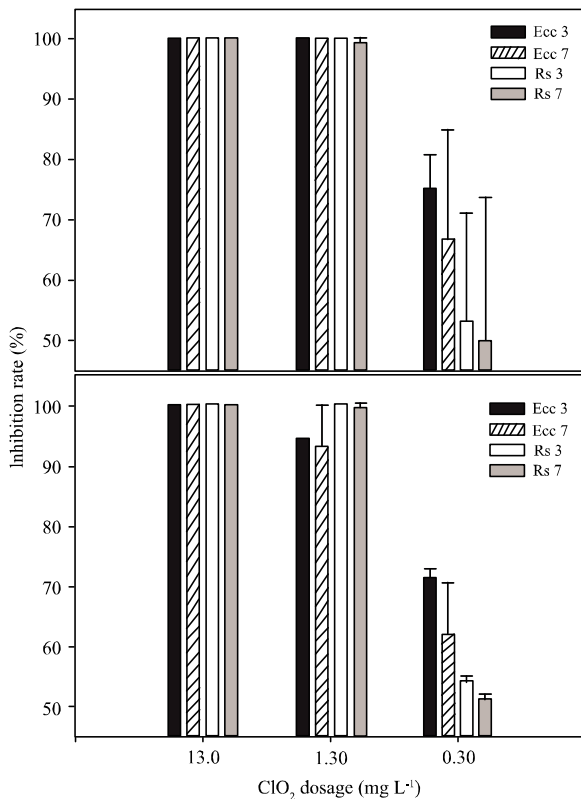


Fig. 2. Inhibition rate of various dosage of ClO₂ solution on (a) sterilized distilled water, and (b) sterilized irrigation water. n = 3.

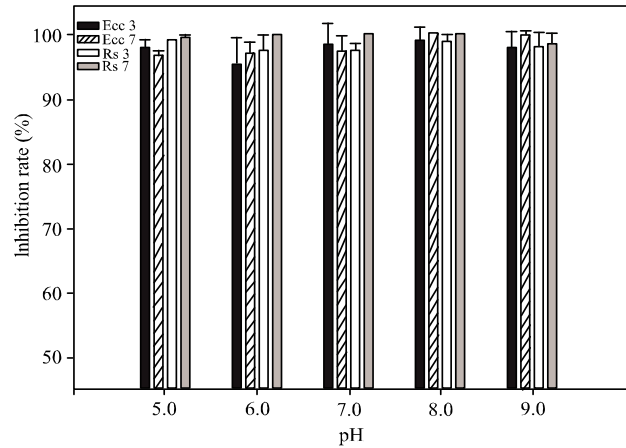


Fig. 3. Inhibition rate of ClO₂ solution at different pH values. n = 3.

[15]. Rober and Reymond further demonstrated that ClO₂ could reduce postharvest pathogen inoculum during handling of the fruits [16]. The US Food and Drug Administration has also approved that ClO₂ is an antimicrobial reagent to wash fruits and vegetables at a residue level of 3 mg L⁻¹ [17]. In this work, the results of bactericidal efficiency of ClO₂ solution are similar to the reports described previously [18-21]. These evidences suggested that ClO₂ disinfectant has potential for application on control of plant disease in irrigation water system.

CONCLUSIONS

In this study, we found that ClO₂ solution at 1.3 mg L⁻¹ could inhibit the growth of phytopathogenic bacteria tested in the present study in the irrigation water. Moreover, the inhibition rate of ClO₂ solution was relatively constant in the pH range of 5 to 9.

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