In vitro Algaecide Effect of Disinfectants on Prototheca zopfii Genotypes 1 and 2

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ABSTRACT. Bovine mastitis due to Prototheca zopfii leads to reduced milk production and is difficult to cure. Therefore, prevention is the best approach and this is best achieved through the use of effective disinfectants. The aim of this study was to evaluate the in vitro algaecide efficacy of conventional disinfectants against strains of P. zopfii genotype 1 and 2. The minimal algaecide concentration (MAC) of alkyldiaminoethylglycine hydrochloride, chlorhexidine, dioxide chlorine, povidone iodine, and sodium hypochlorous acid against 10 isolates and the type strain (SAG2063T) of P. zopfii genotype 1 as well as 10 isolates and the type strain (SAG2021T) of P. zopfii genotype 2 were examined using the micro dilution method. This in vitro study indicated that alkyldiaminoethylglycine hydrochloride, chlorhexidine, povidone iodine and sodium hypochlorous acid, but not dioxide chlorine, are effective against both genotypes of P. zopfii.

KEY WORDS: disinfectants, genotype, mastitis, Prototheca zopfii.

Bovine mastitis due to Prototheca zopfii leads to reduced milk production and thin watery milk secretion containing white flakes. P. zopfii has been classified biochemically and serologically into genotypes 1 and 2, and Prototheca blaschkeae [1, 5, 6]. According to the latest genotypic classification, all isolates from bovine mastitis in Germany and Japan were P. zopfii genotype 2, suggesting that genotype 2 is the principal causative agent of bovine protothecal mastitis [2, 3].

In our previous in vitro study, the sensitivity of P. zopfii genotype 2 isolates was shown to be lower to conventional antimicrobial and antifungal drugs than that of genotype 1 [8]. Currently, bovine mastitis due to Prototheca is a chronic infection with no effective treatments. Therefore, suitable antimicrobial drugs or disinfectants are required to prevent bovine Prototheca mastitis. The aim of this study was to evaluate the in vitro algaecide effects of conventional disinfectants against P. zopfii genotype 1 and 2 strains.

The type strain of genotype 1 (SAG2063T) and the type strain of genotype 2 (SAG2021T) from P. zopfii were used for susceptibility tests.

In addition to the type strains, 10 isolates of P. zopfii genotype 2 from 10 cases of bovine protothecal mastitis in Japan and 10 isolates of P. zopfii genotype 1 from Japanese stock farm were examined [5, 8]. These isolates were previously identified by an 18S rDNA-based genotype-specific PCR assay [2, 3, 5] (Table 1). The disinfectants alkyldiaminoethylglycine hydrochloride, chlorhexidine, dioxide chlorine, povidone iodine and sodium hypochlorous acid were analyzed for their minimal microbicidal concentration (MMC) (M27-A2) [9] using the micro dilution test with some modifications. The minimal algaecide concentration (MAC) of isolates was defined as the lowest disinfectant concentration able to prevent post-exposure algae growth in RPMI 1640 with compound 3-(N-morpholino)propanesulfonic acid (MOPS) [9].

Stock inoculum suspensions were prepared from 3-day-old cultures grown on yeast and mold agar (Oxoid, Ltd., Hampshire, UK) at 37°C, and were adjusted spectrophotometrically to optical densities that ranged from OD 0.8 to 1.0 (OD600) in sterile 0.9% saline. The final concentrations of the inoculums were diluted 1,000 times (2.3 × 10^5 to 3.1 × 10^3 CFU/ml) with RPMI 1640.

The concentrations of the disinfectants were 5 × 10^-9 to 5 µg/ml for alkyldiaminoethylglycine hydrochloride and chlorhexidine, 0.094 to 48 µg/ml for dioxide chlorine (ClO2), 0.012 to 10 µg/ml for povidone iodine and 6 × 10^-9 to 6 µg/ml for sodium hypochlorous acid (NaClO). The concentrations of the disinfectants are according to general use as disinfection at hospitals and dairy farms. After a 48-hr incubation at 32°C, visual reading of wells was performed and the growth of each strain at various concentrations of the disinfectants was recorded. As the control, each of the 22 isolates was grown in drug-free medium and resultant growth recorded. The MAC of an isolate was defined as the concentration of the agent at which 90% of growth was inhibited (MAC90). The experiments were performed in triplicate for each isolate on separate occasions. Agreement was evaluated by MAC endpoint discrepancies of no more than two dilutions.

For all isolates of genotype 1, the MAC90 was 3.13 × 10^-3 µg/ml (range, 5.0 × 10^-4 to 5.0 × 10^-3 µg/ml) for alkyl-
diaminoethylglycine hydrochloride, $3.13 \times 10^{-3}$ µg/ml (range, $2.5 \times 10^{-4}$ to $2.5 \times 10^{-2}$ µg/ml) for chlorhexidine, 60 µg/ml (range, 30 to 120 µg/ml) for dioxide chlorine, 1.17 µg/ml (range: 0.78 to 3.13 µg/ml) for povidone iodine, and 0.069 µg/ml (range: $3 \times 10^{-3}$ to 0.3 µg/ml) for sodium hypochlorous acid.

For all isolates of genotype 2, the MAC$_{90}$ was $1.65 \times 10^{-3}$ µg/ml (range, $5.0 \times 10^{-4}$ to $5.0 \times 10^{-3}$ µg/ml) for alkyldiaminoethylglycine hydrochloride, $1.72 \times 10^{-2}$ µg/ml (range, $2.5 \times 10^{-5}$ to $2.5 \times 10^{-2}$ µg/ml) for chlorhexidine, $51.5 \times 10^{-1}$ µg/ml (range, 15 to 120 µg/ml) for dioxide chlorine, $1.63 \times 10^{-1}$ µg/ml (range: 0.39 to 3.13 µg/ml) for povidone iodine, and 0.12 µg/ml (range: $3 \times 10^{-3}$ to 0.3 µg/ml) for sodium hypochlorous acid.

These in vitro findings indicate that alkyldiaminoethylglycine hydrochloride, chlorhexidine, povidone iodine and sodium hypochlorous acid are effective against both genotypes of *P. zopfii* while dioxide chlorine is not. We recognized that alkyldiaminoethylglycine hydrochloride, chlorhexidine, povidone iodine and sodium hypochlorous acid are algalicidal against *P. zopfii* (data were not shown).

In Japan, sodium hypochlorous acid disinfectant is widely used in dairy farms for the disinfection of floors, walls, utensil and equipment. In the present study, we found that sodium hypochlorous acid should be used at a concentration of $> 0.3 \times 10^{-3}$ µg/ml for dioxide chlorine, $3 \times 10^{-4}$ to 5.0 × $10^{-2}$ µg/ml (range: 30 to 120 µg/ml) for sodium hypochlorous acid. We recognized that sodium hypochlorous acid should be used at a concentration of $> 5.0 \times 10^{-2}$ µg/ml. Moreover, genotype 2 isolates and 7 isolates of genotype 1, including the type strain, were resistant to itraconazole (MIC > 10 µg/ml) [8]. Therefore, disinfectants should be further examined with a view to developing more effective antimicrobial and antifungal drugs for the control of bovine mammary protothecosis in dairy farms.

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REFERENCES


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