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Antibacterial Potential of Biosynthesised Silver Nanoparticles from *Serratia* sp. AQ5-NT39 against *Aeromonas hydrophila*

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Abstract: While the public health community is concerned about the notable rise in *Aeromonas hydrophila* resistance to specific antibiotics, a consistent and rigorous approach is needed to manage this opportunistic pathogen's global presence in the aquaculture sector. It has been demonstrated that *Serratia* sp. strain AQ5-NT39 can biosynthesise silver nanoparticles (AgNPs) through extracellular biosynthesis and exhibit antibacterial activity against pathogenic bacteria. This study will investigate *in vitro* the interaction of biosynthesised AgNPs by *Serratia* sp. strain AQ5- NT39 against *A. hydrophila* using Kirby-Bauer disk diffusion, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). AgNPs AQ5-NT39 demonstrated by forming inhibition zones in the disk diffusion at 8 mm, which showed strong antibacterial activity against *A. hydrophila*. Additionally, the MIC and MBC values at 8 ppm determined the inhibitory and bactericidal effects of the AgNPs. These results underscore the importance of exploring novel nanoparticle applications and their potential antimicrobial implications for addressing bacterial infections in aquatic environments.

Keywords: Antimicrobial, aquaculture, bacteria, green synthesis, nanomaterial.

Introduction

Aeromonas hydrophila, the freshwater pathogen in aquaculture that commonly affects the cultivation of red hybrid tilapia Azzam-Sayuti et al. (2021), has lately emerged as antimicrobial resistance against the conventional antibiotics administered. Siddiqi et al. (2018) stated that many characterised and developed nanoparticles that could exhibit antimicrobial agents and silver nanoparticles (AgNPs) had assumed a significant position owing to their inherent best characteristics. Hence, using AgNPs biosynthesised by *Serratia* sp. strain AQ5-NT39 would be a possible preventive approach in antimicrobial action against the pathogen. The investigation of biosynthesised AgNPs AQ5-NT39 and their antibacterial potential against *A. hydrophila* represents a significant step towards developing effective antimicrobial agents.

Methods

Kirby-Bauer disk diffusion

The antimicrobial activities of AgNPs AQ5-NT39 colloid were tested against the *A. hydrophila* and compared with chloramphenicol and distilled water (dH_2O) (Control-negative) using the agar disk diffusion assay. After lawning the *A. hydrophila* (0.5 McFarland standard) on a sterile Mueller-Hinton agar (MHA) plate, antibiotics and virgin disks (6 mm disks) were placed on the plates. In detail, there are three sterile disks impregnated with each parameter: i) 30 µL of AgNPs AQ5-NT39 colloid, ii) chloramphenicol (30 µg) and iii) 30 µL of dH_2O. The plates were incubated aerobically at room temperature and each parameter's inhibition zones were measured (mm) [zone of inhibition ± SEM] after 18 h.

Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined using the two-fold serial dilution of microdilution assay. Briefly, the strain was prepared based on 0.5 McFarland's standard and diluted to 5 x 10^5 CFU/mL in Mueller-Hinton broth (MHB). Chloramphenicol dilutions were used as the control. After incubation, all broths were observed and recorded for their absorbance reading at 600 nm (OD_{600}). The MIC endpoint was identified as the minimum concentration of AgNPs AQ5-NT39 and chloramphenicol necessary to prevent visible bacterial growth. The two-way ANOVA (post hoc Tukey's test) ($p < 0.05$) was applied to find statistically significant differences in this analysis. To determine the Minimum Bactericidal Concentration (MBC), samples from all the wells of MIC that displayed visible negative growth in the MIC assay were swabbed on MHA agar plates and incubated. From the observation, the lowest AgNPs AQ5-NT39 and chloramphenicol concentration that showed the lowest presence of *A. hydrophila* was labelled as the MBC endpoint, which proves that about 99.9% of the bacterial population is being terminated.

Result and Discussions

Kirby-Bauer disk diffusion

AgNPs AQ5-NT39's antibacterial ability specifically refers to disk diffusion against the *A. hydrophila*. By employing the biological synthesis approach, optimised colloid AgNPs AQ5-NT39 (30 µL) achieved an 8 mm zone of inhibition against *A. hydrophila*, demonstrating the potency of biosynthesised AgNPs. Many theories emphasise that the size and form of the nanoparticles play a significant role in their antibacterial effect, even though the precise mechanism of action of AgNPs is yet unknown. Against this pathogen, chloramphenicol displayed the maximum zone of inhibition, measuring 32 mm. The MIC and MBC assays were used to evaluate further AgNPs AQ5-NT39, which exhibits a considerable level of bacterial inhibition and to demonstrate its bioactivity.

Determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Using biosynthesised AgNPs AQ5-NT39 and chloramphenicol against *A. hydrophila*, MIC values were determined, as shown in **Fig. 1.** Following incubation, AgNPs AQ5-NT39 exhibited MIC values of 8 ppm. In contrast, chloramphenicol's MIC value for *A. hydrophila* was found to be 0.5 ppm.

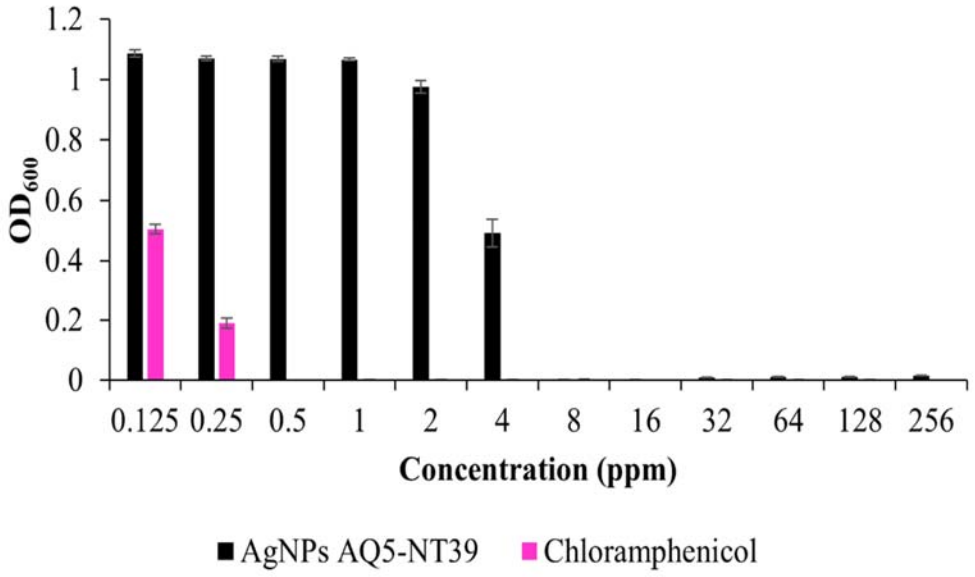

Fig. 1. OD_{600} determines the MIC of AgNPs AQ5-NT39 and chloramphenicol against aquatic pathogenic *A. hydrophila*.

The samples labelled 8 to 256 ppm for AgNPs AQ5-NT39 and 0.5 to 256 ppm for chloramphenicol displayed low absorbance readings at OD_{600}, indicating minimal to virtually non-existent bacterial growths against *A. hydrophila* at 5×10^5 CFU/mL bacterial concentration, respectively (El-Houseiny et al., 2021). The two-way ANOVA showed significant variations between the concentration (ppm) ($F_{11,48}$ = 1,359; $p < 0.0001$) and both antimicrobial agents used ($F_{1,48}$ = 7,989; $p < 0.0001$). The interaction between the concentration (ppm) and antimicrobial agents used is considered significant ($F_{11,48}$ = 805.5; $p < 0.0001$) . MBC was carried out in this investigation for samples in MIC with no apparent turbidity, indicating no bacterial growth (Kyung et al., 2020). By streaking the samples onto MHA plates, MBC analysis was performed on samples with concentrations from 0.5 ppm to 256 ppm. The MBC value for AgNPs AQ5-NT39 against *A. hydrophila* after 24 h of incubation was 8 ppm, matching the MIC value. Meanwhile, no growth was seen for samples 1 to 256 ppm, confirming the bactericidal effect of chloramphenicol against *A. hydrophila*. Further, bacterial growth was seen for samples with a concentration of 0.5 ppm, indicating the bacteriostatic impact of chloramphenicol. Since smaller nanoparticles have stronger antibacterial activity, the shape and size of the AgNPs obtained after optimisation were principally responsible for the AgNPs sample's efficiency against *A. hydrophila*. Although it has a broad spectrum of activity against Gram-positive and Gram-negative bacteria, chloramphenicol is a bacteriostatic agent but is but may be bactericidal in high concentrations or when used against highly susceptible organisms.

Conclusion

The *in vitro* antibacterial action of the biosynthesised AgNPs AQ5-NT39 on the freshwater pathogen *A. hydrophila* has exhibited potential antibacterial activity, as evidenced by the inhibition of bacterial growth during Kirby-Bauer disk diffusion that showed an 8 mm zone of inhibition. This antibacterial effect was further supported by determining the MIC and MBC, representing 8 ppm MIC/MBC.

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