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Biosynthesis of Silver Nanoparticle by *Seratia* AQ5-NT39 for Antibacterial Screening towards *Streptococcus agalactiae*

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Abstract: Aquaculture industry is currently facing many obstacles in meeting the demands and one of the main factors is disease outbreaks in fish farms. Specifically among the majority second most cultivated fish in Malaysia, red hybrid tilapia fish farms due to it prominent causative agent, *Streptococcus agalactiae*. The overuse of antibiotics to control these infections has led to the emergence of antimicrobial resistance, necessitating the search for alternative treatments. This study explores the potential of silver nanoparticles (AgNPs) synthesized by the marine bacterium Serratia sp. AQ5-NT39 as an antibacterial agent against S. agalactiae. AgNPs were biosynthesized through the extracellular production by *Serratia* sp. AQ5-NT39, leveraging the bacterium's natural ability to tolerate heavy metals. The antibacterial properties of these biosynthesized AgNPs were evaluated using in vitro methods, the Kirby-Bauer disk diffusion test. The AgNPs exhibited an inhibition zone of 3.67±0.29 mm compared to the positive control with a zone of inhibition 4.97±0.01 mm. This research demonstrates the promising potential of biosynthesized AgNPs from *Serratia* sp. AQ5-NT39 as a sustainable alternative to antibiotics in the aquaculture industry. Further studies will focus on optimizing the synthesis and application of AgNPs to enhance their antibacterial efficacy and ensure their safety for widespread use.

Keywords: Aquaculture, antibacterial, silver nanoparticles, antibacteria

Introduction

The sustainability of the growing demand for the worldwide aquaculture sector is significantly affected by bacterial disease outbreaks. Common causative bacteria, such as *Streptococcus agalactiae, Streptococcus iniae, Aeromonas hydrophila*, and *Vibrio alginolyticus*, are major sources of infections among fish. These pathogens particularly affect Oreochromis spp. (red hybrid tilapia), the second most preferred type of fish in aquaculture (Ridzuan et al., 2022).

Despite their high tolerance to environmental changes, red hybrid tilapia farms have faced bacterial outbreaks since 2000. A notable recent outbreak occurred in Selangor, Malaysia, in January 2020, due to a co-infection of *A. hydrophila, S. agalactiae*, and tilapia lake virus (TiLV) (Samat et al., 2021). Among these outbreaks, streptococcal disease caused by S. agalactiae is the most predominant and concerning, not only in Malaysia but globally (Wei et al., 2016).

Traditionally, antibiotics have been used to manage these bacterial infections. However, the overuse and misuse of antibiotics have led to the rise of antimicrobial resistance (AMR), posing a significant threat to both aquaculture and public health. As a result, there is an urgent need for alternative antibacterial treatments.

One promising alternative is the use of silver nanoparticles (AgNPs) as they have demonstrated potent antibacterial properties against a wide range of pathogens, including *S. agalactiae* (Bruna et al., 2021). Recent studies have shown that AgNPs synthesized by marine bacteria such as *Serratia* sp. AQ5-NT39 can effectively combat bacterial infections in aquaculture. The biosynthesis of AgNPs by *Serratia* sp. AQ5-NT39 not only leverages the natural properties of these nanoparticles but also provides a sustainable and environmentally friendly approach to disease management (De Silva et al., 2020).

Further research is ongoing to characterize and optimize the synthesis of these AgNPs, with the aim of enhancing their antibacterial efficacy and ensuring their safety for use in aquaculture. This innovative approach holds great potential for reducing the reliance on traditional antibiotics and mitigating the impact of bacterial diseases on the aquaculture industry.

Methods

Biosynthesis and Purification of AgNPs of AQ5-NT39

The AgNPs for the study are biosynthesised from the marine bacteria *Serratia* sp. strain AQ5-NT39, which was obtained from Eco-Remediation Laboratory, Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. The biosynthesis was conducted through the mixture of the cell-free supernatant of the bacterial culture and silver nitrate (AgNO3) solution, followed by several purification steps.

Isolation of Serratia sp. strain AQ5-NT39 Pure Colonies

An amount of 200 µL of *Serratia* sp. strain AQ5-NT39 culture stored in glycerol stock was subcultured into 10 mL of sterile Zobell marine broth and incubated at room temperature in an incubator shaker for 24 h. A loopful of the *Serratia* sp. strain AQ5-NT39 culture was then placed on the prepared Zobell marine agar plate, and four-quadrant streaking was performed. The agar plates were then incubated overnight at 37℃. This experiment was conducted according to Cappuccino & Welsh (2018) with slight modifications.

Biosynthesis of AgNP of AQ5-NT39

The prepared overnight *Serratia* sp. strain AQ5-NT39 culture was centrifuged at 7000 rpm for 10 mins at 4℃ to separate the cells. The cell-free supernatant was mixed with an equal volume of 0.5 M of silver nitrate solution and was incubated for 9 h at room temperature in an incubation shaker at 250 rpm at dark conditions because the AgNPs are sensitive to light (Singh et al., 2016).

Purification of AgNP of AQ5-NT39

Post incubation, the biosynthesised AgNPs mixture of AQ5-NT39 was filtered using a glass filter funnel covered with a Whatman filter paper. Secondary filtration of the mixture was conducted using about 0.1 µm syringe filter to ensure that the solution consists of only nano-sized particles. As the final purification step, the biosynthesised AgNPs were then sonicated for 3 h to break the possible biofilms produced during the biosynthesis and avoid agglutination of the AgNPs. These steps were adapted and modified according to Kabeerdass et al., (2021).

Kirby-Bauer Disc Diffusion Test

This experiment was done according to Kung et al., (2020), A sterile cotton was dipped into an overnight culture of S. agalactiae with the absorbance adjusted to 0.1 at 600 nm (OD600), equivalent to 0.5 McFarland standard, and swabbed against the prepared MHA plates. Prepared discs dipped with the various controls and the biosynthesised AgNPs from AQ5-NT39, respectively, were placed over the agar surface using sterile forceps. The three discs that comprised the biosynthesised AgNPs, distilled water, and commercial ampicillin were gently pressed onto the agar plates to ensure adherence to the surface of the agar. These methods were repeated in triplicates. The MHA plates were incubated in an inverted position for 20 h at room temperature (27˚C). The agar plates were examined for the zone of clear inhibition surrounding each disc.

Results and Discussion

In vitro Antibacterial Testing

In vitro antibacterial testing of the biosynthesised AgNPs of AQ5-NT39 involves the Kirby-Bauer disc diffusion test against *Streptococcus agalactiae*. These tests were conducted to ascertain its antibacterial efficacy of the respective AgNPs to be as equal to or better than the frequently used commercial antibiotics.

Raw colloids of AgNPs were prepared after a 9 h incubation of the mixture of *Serratia* spp. strain AQ5-NT39 bacterial culture supernatant mixed with 0.5 M of silver nitrate (AgNO3) solution at 50°C which was filtered with 0.1 nm syringe filter. The prepared raw colloidal solution served as the test sample to verify its potential antibacterial properties in the initial Kirby-Bauer disc diffusion test against *S. agalactiae*.

Sterile discs were soaked with the raw colloids of the AgNPs of AQ5-NT39 and distilled water, used as the negative control respectively. Additionally, a commercially available ampicillin disc that quantifies 10 µg was served as the positive control. The zone of inhibition (ZOI) of the test sample and the control were measured using a ruler and tabulated in Table 1.

Table 1 Mean values of zone of inhibition of AgNPs of AQ5-NT39, ampicillin and distilled water against *S. agalactiae* conducted in triplicates post 20 h incubation.

Samples	Zone of Inhibition (mm)
AgNPs of AQ5-NT39	3.67±0.29
Ampicillin	4.97±0.01
Distilled Water	0

Note: All values are expressed as mean ± standard deviation.

Although the test sample exhibited a lower ZOI of 3.67±0.29 mm compared to the positive control with a ZOI of 4.97±0.01 mm, the ability of the AgNPs of AQ5-NT39 to showcase antibacterial potential, especially when compared to the negative control suffices the aim of this preliminary Kirby-Bauer antibiotic sensitivity testing against the fish pathogen.

Conclusion

The synthesized of silver nanoparticles (AgNPs) by Serratia sp. has demonstrated antibacterial properties against *Streptococcus agalactiae*. Further studies will focus on characterizing and optimizing these reactions.

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