http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS

Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.90.99

Research Article
Antiviral Properties of *Streptomyces tuirus* DBZ39 Mediated Gold Nanoparticles Against *Bluetongue virus*

Bi Bi Zainab Mazhari

Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Kingdom of Saudi Arabia

Abstract
Background and Objective: The proposed study involves the approach from the point of anti-viral activity of gold nanoparticles against the *Bluetongue virus*. Among viral diseases, Bluetongue is regarded as an economically scouring disease. Neither a vaccine nor an antiviral drug is available for the prevention or treatment of this disease. The antiviral activity of gold nanoparticles synthesized by a novel isolate of *Streptomyces tuirus* DBZ39 is the breakthrough of the study. *Streptomyces tuirus* DBZ39, a novel isolate obtained from alkaline soil was proved to be efficient actinomycetes, for the extracellular synthesis of gold nanoparticles. **Materials and Methods:** An upstream bioprocess was optimized and developed for the synthesis of controlled size gold nanoparticles with solitary mono dispersal pattern in aurum chloride solution. The characterization and confirmation of gold nanoparticles were illustrated by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray Analysis (EDAX) and Fourier Transmission Infrared Radiation Analysis (FTIR). **Results:** Biomass size of 3 g, substrate concentration of 1 mM, pH of 8.5 and temperature of 45°C were observed as optimum conditions for the synthesis of 15-24 nm size gold nanoparticles. The *Bluetongue virus* (BTV) which belongs to the genus Orbivirus in the family Reoviridae with 26 serotypes is an etiological agent of infectious and non-contagious Bluetongue disease of main sheep and several other domestic animals. **Conclusion:** Gold nanoparticles for the 1st time, at a higher concentration of 1:64 dilutions revealed a very promising and novel antiviral property against the *Bluetongue virus*.

Key words: *Streptomyces*, gold nanoparticles, antiviral, *Bluetongue virus*, the etiological agent

Citation: Mazhari, B.B.Z., 2022. Antiviral properties of *Streptomyces tuirus* DBZ39 mediated gold nanoparticles against *Bluetongue virus*. Pak. J. Biol. Sci., 25: 90-99.

Corresponding Author: Bi Bi Zainab Mazhari, Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Kingdom of Saudi Arabia

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The study of fundamental principles of molecules and structures with at least one dimension roughly between one to hundred nanometers is Nanoscience whereas the application of these nanostructures into useful nanoscale devices is nanotechnology[1,2]. Nanoparticles play a vital role in the development of Nanoscience and nanotechnology to serve mankind due to their novel properties such as magnetic, optical, electronics, electrical, catalytic, etc. Nanoparticles offer unique approaches to control a wide variety of biological and environmental processes which have a successful impact on biology and the environment[3,4]. Now a day, researchers are making efforts in integrating nanoparticles in biology by using microbes for the production of metal ions called 'nanofactories'[5]. The increasing demand for gold in many applications leads to the growing need for cost-effective as well as to implement green chemistry in the development of gold nanoparticles[6,7]. Gold nanoparticles have attracted significant interest because of their surface bioconjugation, remarkable Plasmon resonance, optical properties, chemical stability and non-toxicity[8]. Synthesis of nanoparticles using toxic chemicals limits their application. Therefore, there is a need for the development of clean biocompatible, non-hazardous and eco-friendly methods for synthesis[9].

The exploitation of biological systems for the synthesis of gold nanoparticles was a safer and alternative method against physical and chemical methods. The use of microbial cells for the synthesis of gold nanoparticles was considered a novel and green approach. Biological agents like bacteria, fungi, actinomycetes, yeast, algae and plants were used as a wide range of resources for the synthesis[9-11]. In actinomycetes also reduction of metal ions occurs on the surface mycelia along with cytoplasmic membrane leading to the formation of nanoparticles[12].

The antibacterial property of the metal nanoparticles has been explored extensively but the antiviral properties of nanoparticles remain an undeveloped area. The disease caused by the viruses presents challenging problems with worldwide social and economic implications. The most challenging step is developing an antiviral drug that can target a virus and maintain the host cell viability[13]. Gold nanoparticles have been explored for their anti-HIV activity[14,15], *Hepatitis B virus*[16,17], *Respiratory syncytial virus*[18], *Herpes simplex virus* type 1 and 2[19], *Monkeypox virus*[20], *Influenza virus*[21] and *Tacaribe virus*[22]. The expected mechanism of the antiviral gold nanoparticle is the multiple ligands create a high local concentration of binding molecules which can assist the targeted biological interaction. The functionalized group of gold nanoparticle enhances the antiviral properties and help in combating viral infection. Bluetongue is an infectious, non-contagious arthropod-borne viral disease, principally of sheep but many domestic and wild animals like cattle, buffalo, goat, camel and wild ruminants were also affected. Among viral diseases, Bluetongue is regarded as one of the most economically scouring diseases of sheep. Due to its socio-economic impact, involvement with many species of animals and rapid spread across the continent, it is enlisted in the 'multi species disease list' by the Office International Des Epizooties (OIE). Control of Bluetongue disease is very difficult because of the large number of potential host and virus serotypes[23].

The present investigation was intended to synthesize controlled size, extracellular gold nanoparticles by *Streptomyces tuirus* DBZ39 in an optimized bioprocess. Further, the antiviral property of gold nanoparticles against the *Bluetongue virus* was detected.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Virology, SV University and A-DBT Research Laboratory, Gulbarga University, India, from June, 2019 to December, 2020.

Submerged bioprocess for the synthesis of extracellular gold nanoparticles: *Streptomyces tuirus* DBZ39 obtained from alkaline soil was employed for the extracellular synthesis of gold nanoparticles as per the standard protocol prescribed[1,9,24]. A loopful of 3 days old test culture was inoculated into starch casein broth individually and incubated at 40°C for 5 days on a shaker (200 rpm). After the period of incubation, the broth cultures were centrifuged at 8000 rpm for 20 min at 20°C. The biomass obtained was washed 2-3 times with sterile distilled water and then suspended in aurum chloride solution. The biomass suspended solution was kept for incubation at 40°C on a shaker (200 rpm) for 3 days. Visual observation for the development of colour and UV-vis absorbance patterns for the detection of gold nanoparticles were recorded.

Standardization of submerged bioprocess: Synthesis of gold nanoparticles by an efficient isolate *Streptomyces tuirus* DBZ39 was carried out in the submerged system as per the procedure mentioned earlier. The submerged bioprocess was standardized by optimizing important factors namely inoculums size, substrate concentration, pH and temperature. The different range of inoculum size from 1-4 g, substrate concentration from 0.5-2.5 mM, pH from 7.0-9.0 and temperatures from 30-50°C were assessed and synthesis was carried out at optimum conditions.

Characterization of gold nanoparticles: The extracellular gold nanoparticles synthesized by *Streptomyces tuirus* DBZ39 were characterized to determine size, shape and dispersion[25] by following scanning and transmission electron microscopy. The presence of gold nanoparticles was detected by energy dispersive analysis of X-rays-EDAX analysis[25]. The binding of nanoparticles with specific proteins was analyzed by Fourier Transmission Irradiation-FTIR technique[26].

Detection of antiviral property: The antiviral property of gold nanoparticles was carried out as per the standard protocols, prescribed in culture of animal cells[27,28], at the research laboratory, Department of Virology, S.V. University, Tirupati. The protocols followed, in brief are as follows.

Cell lines and culture media: BHK-21 clone 13 cell lines at 46th passage level was obtained from the Molecular Virology Laboratory, Indian Veterinary Research Institute, Bengaluru. Glasgow's Modified Eagle's Medium (DMEM) (M/s Hi media, Mumbai, Cat No. AT007) was used for the maintenance and propagation of BHK-21 cells[29].

Propagation cell lines: Confluent monolayers of BHK-21 cell lines were washed with Ca^{++} and Mg^{++} Free Phosphate Buffer Saline (CMF-PBS) with pH 7.2. One mL of pre-warmed 1×TVG was added to each monolayer in a 25 cm^2 tissue culture flask and spread over the monolayer (Narsimha, 2012). After 1 min, TVG solution was discarded and 5 mL of growth medium was added and mixed thoroughly by repeated pipetting till a uniform single-cell suspension was obtained. Then, the cell suspension was diluted with a growth medium and seeded in fresh flasks at a split ratio of 1:3. The flasks were incubated at 37°C in CO_2 incubator at a 5% CO_2 tension level. Flasks were observed every 24 hrs under the inverted microscope for the formation of a confluent monolayer and were used for virus infection[29].

Propagation of *Bluetongue virus*: Confluent BHK-21 monolayer was washed with 1×CMF-PBS, followed by washing with maintenance medium for adaptation. Then, pre-diluted virus samples with chilled MEM at a concentration of 2.6×10^5 $TCID_{50}$ (to remove the antibodies) were added to the monolayer and incubated at 37°C for 1 hr for viral adsorption. Contents were removed and the 5 mL of maintenance medium was added to the monolayer and incubated at 37°C until 90-100% CPE developed. The sequential changes in the infected monolayer were observed at different time intervals under an inverted microscope. Virus un-inoculated monolayers were maintained as controls[30].

Cytopathogenicity and antiviral property: The initiation of cytopathic effect was observed as early as 24 hrs post-infection with initial rounding and detachment of cells. A tissue culture plate (96 well) was seeded with BHK-21 cell lines at a concentration of 5000 cells/well containing DMEM medium with 1% FCS. After the formation of the confluent monolayer, the medium was changed and test compounds were added at different concentrations. Further, the cell cytotoxicity induced by nanoparticles was examined and recorded. At a minimum concentration (1:64 dilutions), where nanoparticles were not causing any cytotoxicity was selected for antiviral evaluation.

RESULTS AND DISCUSSION

Streptomyces tuirus DBZ39 in (Fig.1a and b) isolated from alkaline soil was proved to be the most efficient isolate

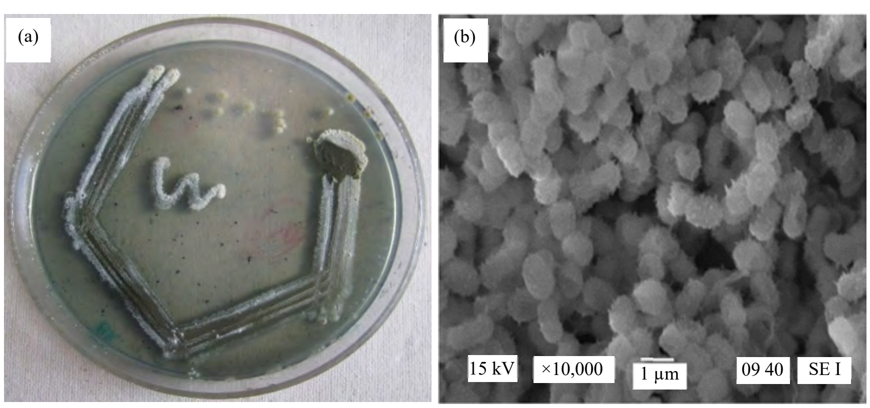

Fig. 1(a-b): (a) Isolate and (b) Electron microscopic sporulation pattern of *Streptomyces tuirus* DBZ39

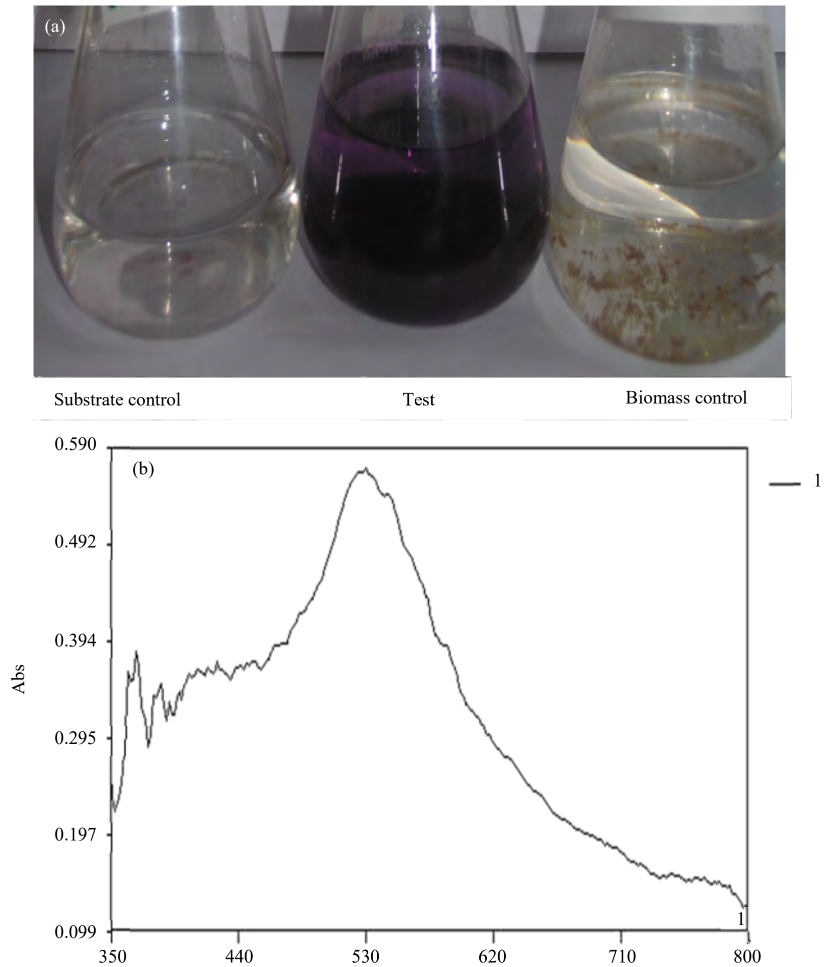

Fig. 2(a-b): (a) Visual observation and (b) UV-vis spectra of extracellular gold nanoparticles synthesized by *Streptomyces tuirus* DBZ39
x-axis: Wavelength

for the extracellular synthesis of gold nanoparticles. Development of deep purple colour showed in Fig. 2a in treated solution when compared to substrate and biomass control solutions indicates the synthesis of gold nanoparticles by *Streptomyces tuirus* DBZ39. The presence of extracellular gold nanoparticles in the solution was confirmed by UV-vis spectrophotometric analysis in Fig. 2b, exhibiting the maximum peak recorded at 520 nm[12].

A large number of reports are available on the synthesis of gold nanoparticles by biological entities, especially from plants, bacteria and fungi. However, there are very few reports on the synthesis of extracellular gold nanoparticles by actinomycetes[31,32]. The development of wine red colour in the test solution within 18 hrs reveals more rapid synthesis and strong physiological capability of *Streptomyces tuirus* DBZ39.

An upstream bioprocess for the synthesis of gold nanoparticles was optimized and standardized with 4 important process variables such as inoculum size, substrate concentration, pH and temperature where the wavelength (X-axis) vs. Absorbance in form of optical density (Y-axis). Inoculum size of 3 g with 1.4 Au optical density at 550 nm in Fig. 3a, substrate concentration of 1 mM with 0.8 Au optical density at 550 nm in Fig. 3b, pH 8.5 with 1.5 Au optical density at 550 nm in Fig. 4a and temperature 4.5°C with 1.2 Au optical density at 550 nm in Fig. 4b were proved to be optimum for the quantity and quality enhanced production of gold nanoparticles.

A strategic approach for the synthesis of extracellular gold nanoparticles is an important criterion to obtain highly controlled size gold nanoparticles. The integration of various process variables as mentioned above for the extracellular

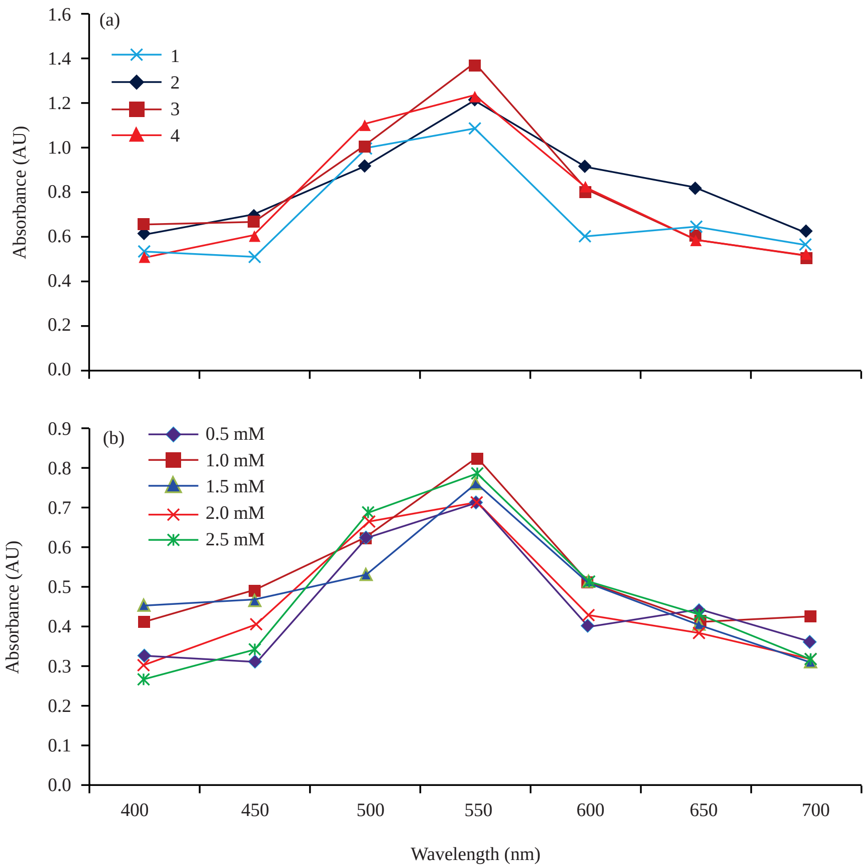

Fig. 3(a-b): UV-vis absorption spectra of gold nanoparticles synthesized by *Streptomyces tuirus* DBZ39 at different (a) Biomass and (b) Substrate concentrations

synthesis of gold nanoparticles by an isolate of actinomycetes, *Thermomonospora* sp. was accounted[1]. Similar reports on novel alkalo thermophilic actinomycetes, *Thermomonospora* sp. isolated from self-heating compost was reported to have pH 9.0, temperature 50°C and 1 mM substrate concentration as optimum conditions for the extracellular synthesis of gold nanoparticles. It was also reported that the use of extreme biological conditions in the synthesis could be a contributory factor in the size and mono dispersal control using actinomycetes as the biological source. The characteristic properties of any nanoparticles are very important and critical from the point of their applications in various fields of biotechnology. Relatively, in the present investigation, conditions such as pH 8.5 and temperature 4.5°C, lead the novel to isolate *Streptomyces tuirus* DBZ39, synthesized a controlled size gold nanoparticle.

The scanning reveals small to medium size nanoparticles with 24 nm in size and spherical shown in Fig. 5. However, the transmission electron microscopy reveals that, a uniform distribution pattern of gold nanoparticles with solitary mono dispersion in the solution in Fig. 6.

The spectrum of EDAX confirms the purity of gold nanoparticles, whereas the spectrum of FTIR exhibits the binding of gold nanoparticles to specific proteins. The Energy Dispersive X-ray Analysis (EDAX) demonstrated the presence of 24 nm size gold nanoparticles in the solution exhibiting the highest peak in the spectrum at gold (Au). The maximum peak obtained with particular metallic element gold (Au), confirms the purity and also the concentration of the element in Fig. 7a. FTIR spectroscopy was employed to detect changes to characteristics of amides bands in gold nanoparticles. FTIR spectrum has shown the presence of 4 peaks 2645.81, 2123.05, 1636.55 and 712.21 in Fig. 7b. Wavenumber between 2645.81 and 2123.05 cm^{-1} indicates hydrogen bond lengths whereas the peak at 1636.55 cm^{-1} corresponds to the N-H bond of primary amines due to carbonyl stretch. Amide 1 is the most intensive absorption band in protein. The absorption bands at the 1636.55 cm^{-1} regions were assigned for the amide bands which was found in the amide linkages of proteins in gold nanoparticles

Synthesis of metal oxide nanoparticles by *Streptomyces* sp. for the development of antimicrobial

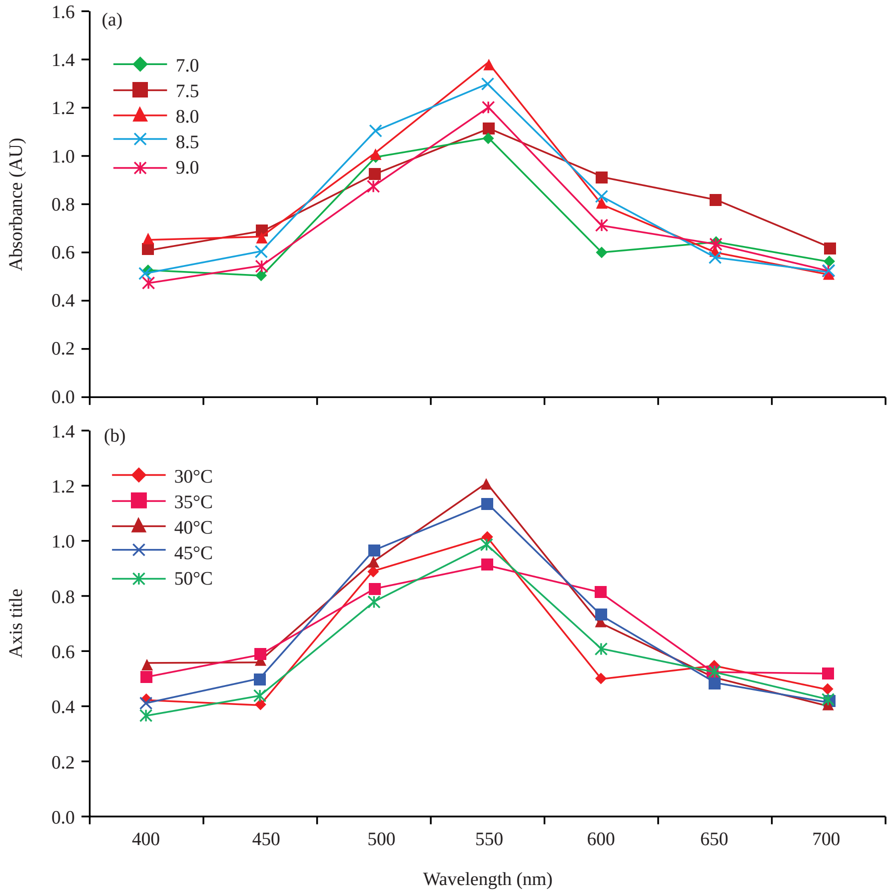

Fig. 4(a-b): UV-vis absorption spectra of gold nanoparticles synthesized by *Streptomyces tuirus* DBZ39 at a different range of (a) pH and (b) Temperature

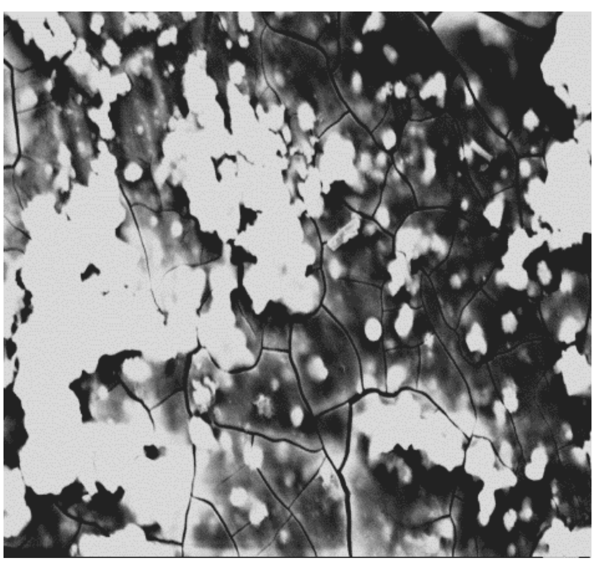

Fig. 5: Scanning electron micrographic features of gold nanoparticles synthesized by *Streptomyces tuirus* DBZ39

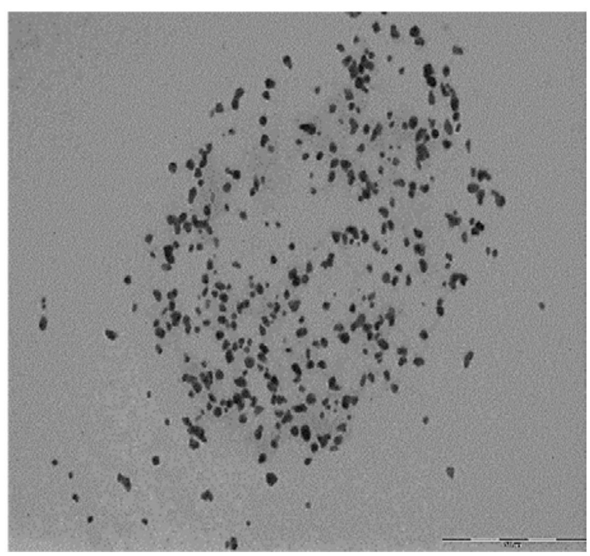

Fig. 6: Transmission electron micrographic features of gold nanoparticles synthesized by *Streptomyces tuirus* DBZ39

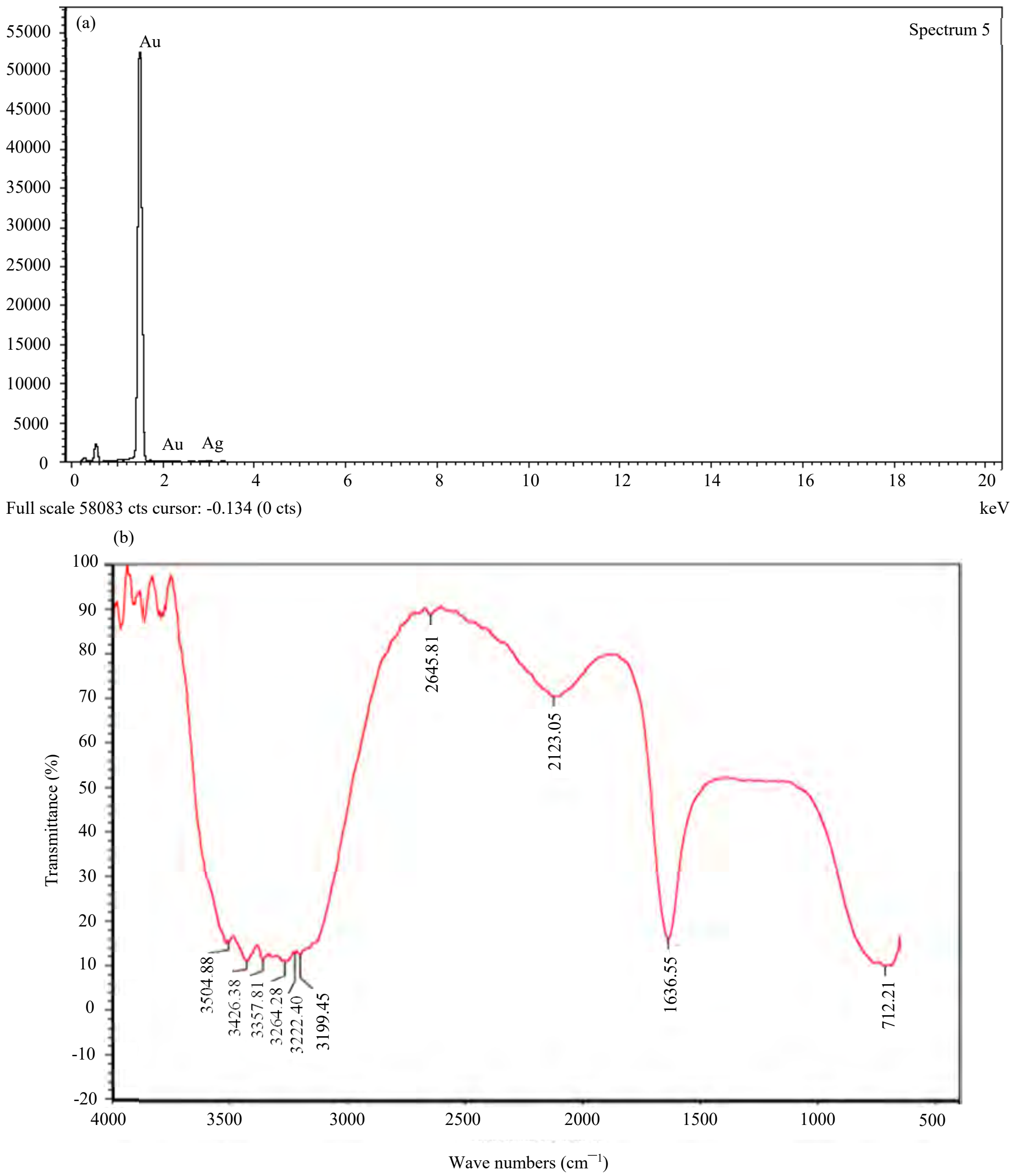

Fig. 7(a-b): (a) EDAX and (b) FTIR spectra of gold nanoparticles synthesized by *Streptomyces tuirus* DBZ39

textiles and screening of the potentials with silver and sodium nanoparticles of marine *Streptomyces* sp. for antimicrobial properties against multidrug-resistant bacteria and fungal strains were reported[31,33]. Isolates of actinomycetes preferably strains belonging to the genus *Streptomyces* could be the better potential sources for extracellular synthesis of gold nanoparticles as *Streptomyces* are known for having several potential attributes of both fungi and bacteria.

Exploration of substrates for antiviral properties is an ever-encouraging field of medical biotechnology. The antiviral property of gold nanoparticles against the *Bluetongue virus* was revealed and the inferences drawn are as presented in Fig. 7. Spindle shape and balloon-like bulgy nature is the characteristic feature of normal cells of BHK-21 cell lines. A monolayer of these BHK-21 cell lines in Fig. 8a gets disappeared when infected with the *Bluetongue virus*. The cytopathic effect induced by the *Bluetongue virus* on monolayer cells of BHK-21 is quite clear and exhibited with the formation of hollow space or patches in Fig. 8b by destroying the healthy network of normal monolayer cells. When monolayer of BHK-21 cell lines infected with *Bluetongue virus* treated with the solution of gold

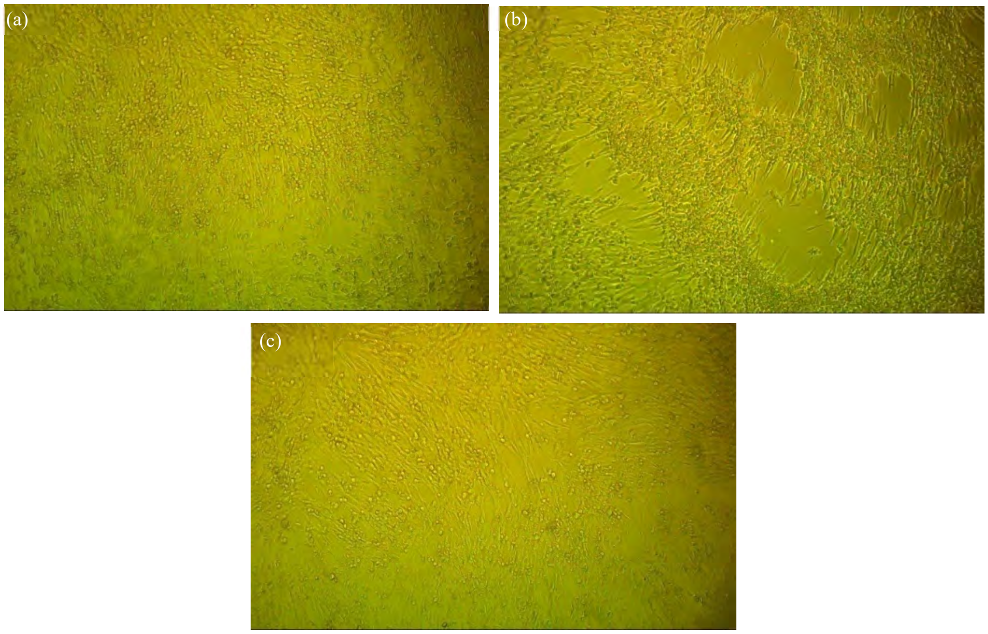

Fig. 8(a-c): Detection of antiviral property of gold nanoparticles against *Bluetongue virus*, (a) Normal cell lines of BHK-21 propagated at 48 hrs (control), (b) Cytopathic effect of *Bluetongue virus* on BHK-21 cell lines at 48 hrs (infected) and (c) Effect of gold nanoparticles on *Bluetongue virus*-infected BHK-21 cell lines at 1:64 dilutions

nanoparticles, the retrieval and regaining of normal and healthy cell networking in Fig. 8c significantly reveals the antiviral property of gold nanoparticles. With the increased concentration of gold nanoparticles at 1:64 dilutions, the cytopathic effect of the *Bluetongue virus* was more inhibited and hence the BHK-21 cell lines regain their healthy morphology and networking. To the best of our knowledge and as per the literature available, the antiviral property of gold nanoparticles against the *Bluetongue virus* was never disclosed earlier and appears to be the first ever-important and critical observation.

Metal nanoparticles have been studied for their antimicrobial potential and have proven to be better antibacterial agents than antifungal agents[34-36]. A very few efforts have been made to determine the interactions of metal nanoparticles and viruses. An account of the potential applications of metal nanoparticles as antimicrobials which includes few antiviral properties is other than the *Bluetongue virus* has been reported. The *in vitro* studies have imparted to understand the possible mechanism by which nanoparticles leads to the induction of apoptosis, inhibition of growth and promotion of differentiation[37]. The main finding of the present investigation is the antiviral activity of gold nanoparticles against the *Bluetongue virus* which has been never disclosed earlier. *Bluetongue virus* causes Bluetongue disease, which is one of the economically scouring diseases of sheep worldwide. Therefore, an attempt was made to target these viruses and gold nanoparticles, at a higher concentration of 1:64 dilutions disclosed a quite promising inhibitory effect on the *Bluetongue virus*.

CONCLUSION

Rapid synthesis of extracellular gold nanoparticles by a novel isolate *Streptomyces tuirus* DBZ39 within 18 hrs of incubation is an important attribute. Controlled size (18-25 nm) synthesis of gold nanoparticles was achieved in a bioprocess with optimized process variables. Detection of antiviral property of gold nanoparticles against *Bluetongue virus* is a significant contribution of the present investigation and first-ever reported novel property.

SIGNIFICANCE STATEMENT

This study discovered the antiviral properties of gold nanoparticles synthesized by *Streptomyces tuirus* DBZ39 that can be beneficial for treating the Bluetongue disease among viral diseases blue tongue is regarded as one of the most economically scouring diseases of sheep. Control of the Bluetongue is very difficult because of the large number of potential host and virus serotypes. Because of this, the present investigation was intended for the welfare of mankind in the treatment of infectious diseases using microbial synthesized extracellular gold nanoparticles in focus against the *Bluetongue virus*, which is a novel approach.

ACKNOWLEDGMENTS

First author is grateful to Prof. Dayanand Agsar, A-DBT Research Laboratory, Gulbarga University, Karnataka and Prof. Sai Gopal, Department of Virology, S.V. University, Tirupati, Andhra Pradesh, India, for grants to carry out the study in the Laboratory.

REFERENCES

1. Sastry, M., A. Ahmad, M.I. Khan and R. Kumar, 2003. Biosynthesis of metal nanoparticles using fungi and actinomycetes. Curr. Sci., 85: 162-170.
2. Klaus-Joerger, T., R. Joerger, E. Olsson and C.G. Granqvist, 2001. Bacteria as workers in the living factory: Metal-accumulating bacteria and their potential for materials science. Trends Biotechnol., 19: 15-20.
3. West, J.L. and N.J. Halas, 2000. Applications of nanotechnology to biotechnology. Curr. Opin. Biotechnol., 11: 215-217.
4. Zandonella, C., 2003. Cell nanotechnology: The tiny toolkit. Nature, 423: 10-12.
5. Sharma, N., A.K. Pinnaka, M. Raje, F.N.U. Ashish, M.S. Bhattacharyya and A.R. Choudhury, 2012. Exploitation of marine bacteria for production of gold nanoparticles. Microbial Cell Factories, Vol. 11. 10.1186/1475-2859-11-86.
6. Villaverde, A., 2010. Nanotechnology, bionanotechnology and microbial cell factories. Microb. Cell Fact., Vol. 9. 10.1186/1475-2859-9-53.
7. Tikariha, S., S. Singh, S. Banerjee and A.S. Vidyarthi, 2012. Biosynthesis of gold nanoparticles, scope and application: A review. Int. J. Pharm. Sci. Res., 3: 1603-1615.
8. Thakkar, K.N., S.S. Mhatre and R.Y. Parikh, 2010. Biological synthesis of metallic nanoparticles. Nanomed. Nanotechnol. Biol. Med., 6: 257-262.
9. Ahmad, A., S. Senapati, M.I. Khan, R. Kumar, R. Ramani, V. Srinivas and M. Sastry, 2003. Intracellular synthesis of gold nanoparticles by a novel alkalotolerent actinomycete, *Rhodococcous* species. Nanotechnology, 14: 10.1088/0957-4484/14/7/323.
10. Musarrat, J., S. Dwivedi, B.R. Singh, Q. Saquib and A.A. Al-Khedhairy, 2011. Microbially Synthesized Nanoparticles: Scope and Applications. In: Microbes and Microbial Technology, Ahmad, I., F.A. Pichtel (Eds.)., Springer, New York, pp: 101-126.
11. Das, S.K. and E. Marsili, 2010. A green chemical approach for the synthesis of gold nanoparticles: Characterization and mechanistic aspect. Rev. Environ. Sci. Bio/Technol., 9: 199-204.
12. Ahmad, A., S. Senapati, M.I. Khan, R. Kumar and M. Sastry, 2005. Extra-intracellular biosynthesis of gold nanoparticles by an alkalotilerant fungus *Trichothecium* spp. J. Biomed. Nanotechnol., 1: 47-53.
13. Namratha, K., K. Byrappa, J. Bai, R.V. Rai, D. Ehrentrant, I.A. Ibrahim and M. Yoshimura, 2013. Antimicrobial activity of silver doped ZnO designer nanoparticles. J. Biomater. Tissue Eng., 3: 190-195.
14. Lara, H.H., N.V. Ayala-Nunez, L. del Carmen Ixtepan Turrent and C.R. Padilla, 2010. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J. Microbiol. Biotechnol., 26: 615-621.
15. Kumar, A., B.M. Boruah and X.J. Liang, 2011. Gold nanoparticles: Promising nanomaterials for the diagnosis of cancer and HIV/AIDS. J. Nanomater., Vol. 2011. 10.1155/2011/202187.
16. Lu, L., R.W.Y. Sun, R. Chen, C.K. Hui and C.M. Ho *et al*., 2008. Silver nanoparticles inhibit *Hepatitis B virus* replication. Antiviral Ther., 13: 253-262.
17. Alipour, E., H. Ghourchian and S.M. Boutorabi, 2013. Gold nanoparticle based capacitive immunosensor for detection of hepatitis B surface antigen. Anal. Methods, 5: 4448-4453.
18. Avilala, J. and N. Golla, 2019. Antibacterial and antiviral properties of silver nanoparticles synthesized by marine actinomycetes. Int. J. Pharm. Sci. Res., 10: 1223-1228.
19. Baram-Pinto, D., S. Shukla, N. Perkas, A. Gedanken and R. Sarid, 2009. Inhibition of *Herpes simplex virus* type 1 infection by silver nanoparticles capped with mercaptoethane sulfonate. Bioconjugate Chem., 20: 1497-1502.
20. Rogers, J.V., C.V. Parkinson, Y.W. Choi, J.L. Speshock and S.M. Hussain, 2008. A preliminary assessment of silver nanoparticle inhibition of *monkeypox virus* plaque formation. Nanoscale Res. Lett., 3: 129-133.
21. Papp, I., C. Sieben, K. Ludwig, M. Roskamp and C. Böttcher *et al*., 2010. Inhibition of *influenza virus* infection by multivalent sialic-acid-functionalized gold nanoparticles. Small, 6: 2900-2906.
22. Speshock, J.L., R.C. Murdock, L.K. Braydich-Stolle, A.M. Schrand and S.M. Hussain, 2010. Interaction of silver nanoparticles with *Tacaribe virus*. J. Nanobiotechnol., Vol. 8. 10.1186/1477-3155-8-19.
23. Unno, Y., Y. Shino, F. Kondo, N. Igarashi and G. Wang *et al*., 2005. Oncolytic viral therapy for cervical and ovarian cancer cells by *Sindbis virus* AR339 strain. Clin. Cancer Res., 11: 4553-4560.
24. Shahverdi, A.R., S. Minaeian, H.R. Shahverdi, H. Jamalifar and A.A. Nohi, 2007. Rapid synthesis of silver nanoparticles using culture supernatants of *Enterobacteria*: A novel biological approach. Process Biochem., 42: 919-923.

25. Vigneshwaran, N., N.M. Ashtaputre, P.V. Varadarajan, R.P. Nachane, K.M. Paralikar and R.H. Balasubramanya, 2007. Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Mater. Lett., 61: 1413-1418.
26. Krishnaraj, C., E.G. Jagan, S. Rajasekar, P. Selvakumar, P.T. Kalaichelvan and N. Mohan, 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf. B: Biointerfaces, 76: 50-56.
27. SungJun, P., H.H. Park, S.Y. Kim, S.J. Kim, K. Woo and G. Ko, 2014. Antiviral properties of silver nanoparticles on a magnetic hybrid colloid. Appl. Environ. Microbiol., 80: 2343-2350.
28. Rai, M., K. Kon, A. Ingle, N. Duran, S. Galdiero and M. Galdiero, 2014. Broad-spectrum bioactivities of silver nanoparticles: The emerging trends and future prospects. Appl. Microbiol. Biotechnol., 98: 1951-1961.
29. Forbes, B.A., A.S. Weissfeld, D.F. Sahm and P. Tille, 2007. Bailey and Scott's Diagnostic Microbiology. 12th Edn., Elsevier Mosby, United States, ISBN-13: 9780323040839.
30. Oves, M., M.A. Rauf, A. Hussain, H.A. Qari and A.A.P. Khan *et al*., 2019. Antibacterial silver nanomaterial synthesis from *Mesoflavibacter zeaxanthinifaciens* and targeting biofilm formation. Front. Pharmacol., Vol. 10. 10.3389/fphar.2019.00801
31. Ghosh, S., R. Ahmad, M. Zeyaullah and S.K. Khare, 2021. Microbial nano-factories: Synthesis and biomedical applications. Front. Chem., Vol. 9. 10.3389/fchem. 2021. 626834.
32. Sayadi, K., F. Akbarzadeh, V. Pourmardan, M. Saravani-Aval, J. Sayadi, N.P.S. Chauhan and G. Sargazi, 2021. Methods of green synthesis of Au NCs with emphasis on their morphology: A mini-review. Heliyon, Vol. 7. 10.1016/j. heliyon.2021.e07250.
33. Derakhshan, F.K., A. Dehnad and M. Salouti, 2012. Extracellular biosynthesis of gold nanoparticles by metal resistance bacteria: *Streptomyces griseus*. Synth. React. Inorg. Metal Org. Nano-Metal Chem., 42: 868-871.
34. Rai, M., A. Yadav and A. Gade, 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv., 27: 76-83.
35. Kim, J.S., E. Kuk, K.N. Yu, J.H. Kim and S.J. Park *et al*., 2007. Antimicrobial effects of silver nanoparticles. Nanomed. Nanotechnol. Biol. Med., 3: 95-101.
36. Basavegowda, N., A. Sobczak-Kupiec, D. Malina, H.S. Yathirajan and V.R. Keerthi *et al*., 2013. Plant mediated synthesis of gold nanoparticles using fruit extracts of *Ananas comosus* (L.) (pineapple) and evaluation of biological activities. Adv. Mater. Lett., 4: 332-337.
37. Lengfelder, E., W.K. Hofmann and D. Nowak, 2012. Impact of arsenic trioxide in the treatment of acute promyelocytic leukemia. Leukemia, 26: 433-442.