CHAPTER V
DISCUSSION AND CONCLUSION

Streptomyces bacteria are the genus of the Actinomycetales order. *Streptomyces* are aerobic, filamentous, Gram-positive bacteria and are commonly found in a variety of environments, especially in soil. *Streptomyces* is a good source of extracellular enzymes and bioactive compounds. *Streptomyces* sp. produces secondary metabolites for clinically important bioactive compounds with antivirals, antitumor, antifungal, antibiotics, and insecticidal properties (Khan et al., 2011; Procópio et al., 2012; Song et al., 2004). The *Streptomyces* has a various colony structure based on spore color, substrate and aerial mycelium development, and diffusible pigment productions (Michael et al., 2012). In this study, *Streptomyces* strain MSK03 and MSK05 were isolated from Sakaerat Environmental Research Station and Botanical Garden at the Suranaree University of Technology, respectively. The phenotypic characteristics, such as colony morphology, sporulation, and pigment, of *Streptomyces* strain MSK03 and MSK05 were identified on ISP2 medium. The identification of bacteria based on phenotypic characteristics was not quite as effective as molecular identification (Zhang et al., 2019).

Thus, the soil isolated MSK03 and MSK05 were identified based on the 16s rRNA gene sequence and phylogenetic connection. The molecular techniques based on 16S rRNA gene sequences were used to confirm the strain of several bacteria (Taylor et al., 2007; Zhang et al., 2019). The 16S rRNA sequencing is widely used for bacteria identification and taxonomic classification. Bacterial identification of the 16S rRNA gene uses approximately 1500 base pair genes located on small subunits in the 30s of prokaryotic ribosomes. The 16S rRNA sequencing has the advantages of speed, cost-effectiveness, and high precision (Case et al., 2007; Kim and Chun, 2014; Reller et al., 2007; Yang et al., 2016). The steps in 16S rRNA sequencing consist of DNA extraction using chemicals, PCR amplification of the target sequence, DNA elution by agarose gel electrophoresis, and follow by target band purification (Antony-Babu et

al., 2017; Takeuchi et al., 1996). For phylogenetic investigations, the 16S rRNA gene is employed to compare different species of bacteria. The comparison of 16S rRNA gene sequences to induce phylogenetic relationships of different bacteria species have been widely used for several decades (Coenye and Vandamme, 2003; Weisburg et al., 1991).

In this research, the 16S rRNA gene of MSK03 shared 99.79% sequence similarity to *S. monashensis* with 100% bootstrap value, while MSK05 shared 99.86% sequence similarity to *S. spectabilis* with 95% bootstrap value. Therefore, soil isolated MSK03 was identified as *S. monashensis,* while MSK05 was identified as *S. spectabilis*. The phylogenetic tree from evolutionary distance data of soil isolates MSK03 and MSK05 was generated by using the neighbor-joining method with 1,000 replications bootstrap analysis. The neighbor-joining method is commonly utilized in the construction of phylogenetic trees because of its excellent accuracy and computational speed in the phylogenetic analysis as demonstrated by computer simulation investigations. The basic idea behind this strategy is to locate pairs of operational taxonomic units (OTUs) that minimize overall branch length at each level of the OTU clustering (Saitou and Nei, 1987).

In recent years, antibiotic-resistant microorganisms have emerged. The development of high-resistance genes has linked to high levels of antibiotic resistance (Hsueh, 2010; Shaikh et al., 2015). Nanotechnology is a rapidly developing field with enormous potential. Nanomedicine offers a good foundation for combating drug resistance (Shaikh et al., 2015). Moreover, nanoparticles are used in a wide range of applications, including agriculture, solar cells, pollution control, waste management, and medicine approaches for anticancer, antitumor, antibacterial activity, and so on (Basu et al., 2015). The particle size of technology at the nanoscale is small particles in nanoscale ranging from 1 to 100 nm (Lee et al., 2020). Nanoparticles are very attractive for a wide variety of biotechnology applications due to their unique chemical and physical properties (Shah et al., 2014). Because of their physical, biological, and chemical characteristics, gold nanoparticles are among the most commonly used metal nanoparticles due to their potential for a wide variety of applications such as water purification, food industry, biological, and medicinal

applications (Menon et al., 2017; Shahzadi et al., 2018; Zhang et al., 2015). Gold metal and gold ionic form have long been recognized to have broad spectrum antibacterial and bactericidal effects (Agnihotri et al., 2013; Zhang et al., 2015; Zhou et al., 2012). The properties of AuNPs depend on morphology and reaction modifications to obtain AuNPs of the desired size, shape, and surface functionality (Shah, Badwaik, and Dakshinamurthy, 2014; Sperling et al., 2008). AuNPs have bio-nanotechnology applications such as sensing for detection and diagnostics, transport of therapeutic agents to the cells, cell imaging, antimicrobial, and so on (Yeh et al., 2012).

Chemical and physical processes, such as chemical, photochemical, and electrochemical reduction, are used to synthesize AuNPs (Kshirsagar et al., 2014; Scaiano et al., 2009; Yang et al., 2011). These methods usually depend on toxic chemicals as reducing or capping agents. The presence of precursor materials may cause the cytotoxicity of healthy cells (Shukla and Iravani, 2018). An environmentally friendly approach to nanoparticle synthesis is expected to overcome the toxicity problem in nanoparticle synthesis. Green synthesis focuses on reducing and eliminating environmentally hazardous processes (Jahangirian et al., 2017; Yang et al., 2011). The green materials, such as biopolymers, enzymes, fungi, bacteria, and plants, were used to synthesize nanoparticles (Lee et al., 2020). Microorganisms are commonly used for the biosynthesis of AuNPs. Actinobacteria isolated from several environments have been identified as potential to synthesize AuNPs. The biosynthesis of AuNPs is available in the intracellular and extracellular of the actinobacteria, including *Streptomyces* (Manivasagan et al., 2016). The biosynthesized AuNPs using intracellular and extracellular *Streptomyces* was illustrated in Table 5.1.

In the synthesis process, the AuNPs exhibit changing colors from light yellow to purple, red, orange, and brown in an aqueous solution. The color change is due to the increase in the size distribution of AuNPs from 1 to 100 nm (Jain et al., 2006). Moreover, the presence of color change is a direct sign of the reduction of Au^{3+} to Au^{0} (Hassanisaadi et al., 2021). Soltani and co-workers reported the color changes from pale yellow to deep red on the AuNPs biosynthesis using *S. fulvissimus* isolate U after incubation at 30°C for 48 h (Soltani et al., 2015). They have also reported a

similar observation of the color changes using *S. microflavus* isolate extracellular after incubation at 28°C for 24 h (Soltani et al., 2016). The color changes from yellow to pinkish purple on the AuNPs biosynthesis using intracellular of *S. viridogens* strain HM10 after incubation at 28°C for 24 h was reported by Balagurunathan et al. (Balagurunathan et al., 2011). In this research, the synthesis of AuNPs used both extracellular and intracellular cell-free supernatants of *Streptomyces* sp. (MSK03 or MSK05) as reducing agents. The color change from light yellow to purple was observed from synthesized AuNPs using extracellular cell-free supernatant of *Streptomyces* spp.

Table 5.1 Biosynthesis of AuNPs using *Streptomyces* spp.

Actinomycete strain	Precursor	Activity	References
S. viridogens HM10	intracellular	antimicrobial	(Balagurunathan et al., 2011)
S. hygroscopicus BDUS 49	extracellular	antimicrobial	(Sadhasivam et al., 2012)
Streptomyces sp.	extracellular	antifungal	(Gopal et al., 2013)
Streptomyces sp LK-3	extracellular	antimalarial	(Karthik et al., 2013a)
Streptomyces sp. NK52	extracellular	anti-lipid peroxidation	(Prakash et al., 2013)
S. fulvissimus isolate U	extracellular	ND	(Soltani et al., 2015)
S. microflavus isolate 5	extracellular	ND	(Soltani et al., 2016)
S. coelicoflavus SRBVIT13	extracellular	anti-hyperglycemic	(Kumar and Rao, 2016)
S. griseoruber	extracellular	catalytic	(Ranjitha and Rai, 2017)
Streptomyces sp. strain NH21	extracellular	antibacterial	(Składanowski et al., 2017)
S. tuirus DBZ39	intracellular	antiviral	(Zainab, 2022)

ND, not determined.

The optical properties of AuNPs are phenomena caused by surface plasmon resonance (SPR). The plasmon band occurs when the frequency of electron oscillations in the conduction band of AuNPs resonates with the frequency of incoming light radiation. The SPR in AuNPs requires a specific frequency range of light radiation, which is commonly observed in the visible regions (Mie, 1908; Shankar et al., 2004). UV–Vis absorption spectrophotometry was used to determine the position of localized surface resonance plasma resonance (LSPR) bands to confirm the color change of the synthesized AuNPs (Sharma et al., 2016). The specific frequency of the surface plasmon resonance peak depends on the shape and size of AuNPs (Huang and El-Sayed, 2010; Link and El-Sayed, 1999). In general, spherical AuNPs have been shown a single plasmon band ranging from 500 to 580 nm (Hu et al., 2020; Kim et al., 2016; Poinern, 2014). Sathish Kumar and Bhaskara Rao (2016) reported the maximum absorbance peak at 540 nm to confirm the presence of biosynthesized AuNPs using *S. coelicoflavus* SRBVIT13. Soltani et al. (2015) reported a strong peak with a maximum absorbance of synthesized AuNPs using *S. fulvissimus* isolate U at 550 nm. In this study, the biosynthesized AuNPs using *Streptomyces* sp. MSK03 and MSK05 showed the highest intensity of the LSPR band for AuNPs at 545 and 530 nm, respectively. The results confirmed the presence of AuNPs synthesized by *Streptomyces* sp. MSK03 and MSK05.

In addition, the increase in the particle size of AuNPs affects the change of the maximum absorption that shows a red shift of wavelength (wavelength increasing). The increase in absorbance wavelength is caused by higher electromagnetic retardation. The intensity of the absorbance peak also demonstrates the higher number of AuNPs. The increase in AuNPs concentration led to an increase in the absorbance intensity (Cui et al., 2012; Link and El-Sayed, 1999; Menon et al., 2017; Shukla and Iravani, 2017; Zhou et al., 2012). In this study, UV-Vis absorbance was used to observe the AuNPs synthesis process. The different incubation times of the AuNPs synthesis process depend on reducing agents. This evidence pointed to the reduction of Au^{3+} to Au^{0} by reducing agents found in extracellular cell-free supernatant of *Streptomyces* sp. MSK03 and MSK05. The AuNPs were synthesized using extracellular cell-free supernatant of *S. monashensis* MSK03 and *S. spectabilis*

MSK05 at 37°C for 72 h. The incubation process takes shorter than the biosynthesis of AuNPs using other reducing agents such as extracellular cell-free supernatant of *S. hygroscopicus* BDUS 49, which has been taken 120 h for the incubation process (Sadhasivam et al., 2012). The biosynthesis of AuNPs using *S. viridogens* HM10 as reducing agents has been taken 120 to 168 h for incubation process (Balagurunathan et al., 2011). The detailed mechanism of the reduction process of Au^{3+} to Au^{0} remains unclear. Several researchers have been proposed the reducing agents of biosynthesized AuNPs such as components like lipids, carbohydrates, nucleic acids, or proteins (Shah et al., 2014). While the reducing agents of biosynthesized AuNPs using *Streptomyces* spp. have been proposed as cell wall reductive enzymes and enzymes on the cytoplasmic membrane (Ahmad et al., 2003; Manivasagan et al., 2016)

The probable organic functional groups of capping, stabilizing, and reducing agents for the synthesized AuNPs can be identified using FTIR analysis. The functional groups of biomolecules that are attached to the surface of AuNPs have the potential to involve the reduction of Au^{3+} to Au^{0} (Dahoumane et al., 2016; Elavazhagan and Arunachalam, 2011; Chithrani et al., 2006; Manivasagan et al., 2016). Sathish Kumar and Bhaskara Rao (2016) reported that the functional groups of proteins such as amide, carboxyl, and hydroxyl groups from *S. coelicoflavus* SRBVIT13 are attached to the AuNPs surface as stabilizing agents. Soltani Nejad et al. (2016) reported the presence of hydroxyl groups, carboxyl groups, and N–H stretching of amine groups in biomass of *S. microflavus* isolate No. 5 that are involved in the AuNPs reduction. The presence of a different functional group corresponding to N-H or O-H, C-H, C=O, C-O, and the metal-ligand stretching frequency from the FTIR spectra of the synthesized AuNPs using *S. griseoruber* was reported by Ranjitha and Rai (2017). It has been suggested the possible compounds with N–H or O–H functional groups for the AuNPs stabilization (Dhas et al., 2014). In this study, FT-IR spectra of the biosynthesized AuNPs using extracellular cell-free supernatant of *Streptomyces* sp. MSK03 and MSK05 showed similar regions of five major peaks: N-H or O-H stretching, C=O stretching or N-H bending, C-N stretching or N-H bending, C-H stretching, and C-H bending or metal-ligand stretching. The FT-IR spectra were identified using the FTIR

database. The absorption peaks of O-H, N-H, and C=O stretching correspond to the functional groups of carbohydrate, amine, and amide, respectively. The other regions of N-H bending, C-H stretching, and C-H bending correspond to the amine functional groups of the protein. These results indicated the probable functional groups of *Streptomyces* sp. MSK03 and MSK05 might be associated with the reduction of Au^{3+} and stabilization of AuNPs. The change in peak intensity or shift in the wavenumber of functional groups from the extracellular-free supernatant to AuNPs describes the class of functional groups involved in the interaction of AuNPs and biomolecules (Wan et al., 2020).

The surface charge or zeta potential of colloidal AuNPs solutions was determined using DLS (Jiang et al., 2009). Zeta potential results describe the surface charge, stability, and aggregation condition of colloidal particles of biosynthesized AuNPs (Graily-Moradi et al., 2020; Mittal et al., 2013; Muthuvel et al., 2014). Typical zeta potential values of surface charge of nanoparticles in colloidal solution range from +100 to -100 mV (Shnoudeh et al., 2019). A large positive or negative zeta potential value indicates that the nanoparticle dispersion is physically stable due to the electrostatic repulsion of individual particles. The zeta potential of the nanoparticles with values less than -25 mV or more than +25 mV usually have high degree of stability. While a low zeta potential value of nanoparticle dispersion will lead to coagulation, flocculation, or aggregation due to Van der Waals interaction, resulting in poor physical stability (Brock, 2004; Clogston and Patri, 2011a; Clogston and Patri, 2011b; Horie and Fujita, 2011; Sapsford et al., 2011; Shnoudeh et al., 2019). Karthik et al. (2013a) reported the zeta potential as 28.6 mV, indicating the stability of biosynthesis of AuNPs using marine actinobacterial in remaining distinct in each liquid. Składanowski et al. reported the zeta potential of -14.5 mV of biosynthesized AuNPs using *Streptomyces* sp. strain NH21, indicating moderate stability of nanoparticles (Składanowski et al., 2017). In this research, the value of zeta potential of MSK03-AuNPs and MSK05-AuNPs was -0.53 mV and -0.14 mV, respectively. The MSK03-AuNPs and MSK05-AuNPs had low zeta potential values, resulting in poor physical stability due to coagulation, flocculation, or aggregation of nanoparticles dispersion.

EDXRF technique was used to confirm the chemical or elemental composition of metal nanoparticles (Shah et al., 2015; Strasser et al., 2010). The electron beam hits the inner electrons shell of the atom, knocking out the inner electrons and resulting in a positively charged electron hole. After that, the electron hole in the vacant position is replaced by another electron from the outer shell. The standard two-dimensional graph of the EDX spectrum represents the ionization energy, while the ordinate represents the counts. It has been reported the optical absorption peak of surface plasmon resonance adsorption in gold nanocrystal at around 2.30 keV (Arunachalam et al., 2013). Sathish Kumar and Bhaskara Rao reported the EDX profile of the biosynthesized AuNPs using marine *S. coelicoflavus* SRBVIT13. The EDX pattern of the sample exhibits signals for O, C, and Au atoms, confirming biomolecular capping on AuNPs surfaces. The EDX profile of the biosynthesized AuNPs using *S. coelicoflavus* SRBVIT13 showed the gold signals at around 2.20, 9.70, and 11.42 keV (Kumar and Rao, 2016). Soltani et al. reported the EDX profile of the biosynthesized AuNPs using *S. fulvissimus* isolate U, which represents the number of AuNPs in the biomass. The EDX profile of the biosynthesized AuNPs using *S. fulvissimus* showed strong signals for AuNPs at different places at 2.20, 9.70, and 11.5 keV (Soltani et al., 2015). In addition, it has been reported the EDX profile of the biosynthesized AuNPs using *S. microflavus* isolate 5 that showed strong signals of AuNPs at 2.20, 9.70, and 11.5 keV (Soltani et al., 2016). In this study, the EDX results confirmed the presence of gold elemental in synthesized AuNPs. The presence of a gold pattern in the MSK03-AuNPs is similar to MSK05-AuNPs (Figure 4.13). The EDX spectra of Au elements were observed at approximately 2.2, 9.7, and 11.5 keV. The other elements, including chloride (Cl), potassium (K), and phosphorus (P), were detected in the colloidal AuNPs due to traces of the cell-free supernatant and the gold salt.

The TEM provides information on the morphology, shapes, and size of nanoparticles (Montes et al., 2011; Schaffer et al., 2009). In this study, The TEM images of MSK03-AuNPs and MSK05-AuNPs were spherical and polygonal. The spherical shape is a general morphology of biosynthesized AuNPs using *Streptomyces* sp. (Manivasagan et al., 2016). For example, biosynthesized AuNPs using *S. viridogens*,

S. griseus, *S. hygroscopicus*, *Streptomyces* sp. ERI-3, *S. microflavus*, and *S. tuirus* DBZ39 had spherical morphology (Balagurunathan et al., 2011; Khadivi et al., 2012; Sadhasivam et al., 2012; Soltani et al., 2016; Zainab, 2022; Zonooz et al., 2012). Menon's review study shown that the size of biosynthesized AuNPs with spherical shape ranges from 1 to 200 nm (Menon et al., 2017). While the size of spherical biosynthesized AuNPs produced by *Streptomyces* sp. has been recorded to range between 5 to 50 nm (Manivasagan et al., 2016; Menon et al., 2017). In this study, the TEM result of MSK03-AuNPs represents the spherical shape with an average size of 23.2 ± 10.7 nm, ranging from 7.1 to 40.0 nm. MSK05-AuNPs represent the spherical shape with an average size of 20.3 ± 9.7 nm, ranging from 3.3 to 40.0 nm. In addition, DLS was used to estimate the particle size distribution of biosynthesized AuNPs using a liquid solution (Mittal et al., 2013; Muthuvel et al., 2014; Wu et al., 2018). In this study, MSK03-AuNPs had an average particle size of 46.34 nm in the hydrodynamic diameter range, with a PDI of 0.268. MSK05-AuNPs have an average size of 23.33 nm and a PDI of 0.465. The PDI of more than 0.1 indicates the polydisperse particle size distributions of MSK03-AuNPs and MSK05-AuNPs (Raval et al., 2019). It has been reported that the particle size observed in the TEM image is smaller than that measured by the DLS analyzer (Muthuvel et al., 2014; Ranjitha and Rai, 2017). Muthuvel et al. reported that the DLS result for AuNPs had an average particle size of around 50 nm, while the TEM image showed the size distribution of AuNPs ranging from 5 to 35 nm with an average size of 32 ± 6 nm (Muthuvel et al., 2014). Ranjitha and Rai reported that the DLS result for AuNPs using *S. griseoruber* to have an average particle size of around 80.9 nm, while the TEM image showed the size distribution of AuNPs range from 5 to 50 nm (Ranjitha and Rai, 2017). They have been proposed that the TEM image measures the particle size of the dried sample, while DLS measures the particle size distribution of the hydrodynamic particle diameter with a dynamic light scattering of AuNPs dispersed in a liquid phase (Muthuvel et al., 2014; Ranjitha and Rai, 2017).

XRD is a common technique for determining the diffraction pattern of compounds. XRD analysis is a widely used to determine the crystal structure of AuNPs (Bennur et al., 2016; Jeffery, 1971; Manivasagan et al., 2016). The diffraction

peaks of MSK03-AuNPs and MSK05-AuNPs were compared to an XRD standard for AuNPs structure Joint Committee on Powder Diffraction Standards reference no. 04-0784 in a database of XRD patterns maintained by the International Center for Diffraction Data. The diffraction peaks of AuNPs were located at approximately 2theta of 38°, 45°, 65°, and 78° (Figure 4.8) which correspond to (111), (200), (220), and (311) reflections of the face-centered cubic (fcc) structure of the standard gold metal (Au^{0}), respectively (Balagurunathan et al., 2011; Cheng et al., 2015; Prakash et al., 2013; Ranjitha and Rai, 2017; Ren et al., 2015; Kumar and Rao, 2016; Soltani et al., 2015). Khadivi Derakhshan et al. reported XRD spectra of the biosynthesis of AuNPs by *S. griseus* and $HAuCl_4$ to represent the AuNPs formation after 48 h. The XRD spectrum of biosynthesized AuNPs using *S. griseus* revealed five intense peaks, 38.27, 44.60, 64.68, 77.55, and 82.35, which correspond to fcc structure of AuNPs (Khadivi et al., 2012). The XRD pattern analysis revealed peaks on the biosynthesized AuNPs using the cell-free supernatant of *Streptomyces sp.* VITDDK3 at 38.10 (111), 44.28 (200), 64.42 (220), and 77.37 (311), corresponding to the diffraction plane of Au^{0} was previously reported by Gopal et al. (Gopal et al., 2013). Sathish Kumar and Bhaskara Rao reported the XRD pattern of biosynthesized AuNPs using *S. coelicoflavus* SRBVIT13. The results of biosynthesized AuNPs using *S. coelicoflavus* SRBVIT13 showed the crystalline nature of AuNPs at 2theta of 38.28, 44.27, 64.65, and 77.66, corresponding to the reflection planes of the fcc phase of AuNPs (Kumar and Rao, 2016).

XANES is an absorption spectroscopy technique that used to describe the local electronic structure of an atom. The use of synchrotron radiation in XANES spectroscopy is an authorized technique for determining the electronic, magnetic, and structural properties of matter. Photon absorption in XANES stimulates an electron from a core state to an empty state. To excite an electron at the core level, the photon energy must be equal to or higher than the binding energy of that level. When the photon energy is scanned, a new absorption channel is opened. XANES is an element-selective technology because absorption edge energy corresponds to core-level energy that is unique to each element. XANES is a powerful technique for studying mineral surfaces and adsorbents on mineral surfaces and characterizing bulk

minerals. The element specificity of XANES spectroscopy and the ability to access comprehensive data in the absence of long-range order are two of its unique features. In many cases, the density of unoccupied electronic states in a process is demonstrated to be closely related to the X-ray absorption spectra. XANES can provide a detailed picture of local electrical structure of an element (Bianconi, 1980; Henderson et al., 2014). X-ray absorption fine structure spectroscopy at the Au L3 and L2 edges indicated the formation of AuNPs *in situ*. The electronic and dispersion characteristics of AuNPs during their growth were analyzed using a series of X-ray absorption near-edge structure spectra at both edges (Ohyama et al., 2011). Konishi et al. reported the different profile energy of XANES spectra between Au^{0} (Au foil), Au^{3+} ($HAuCl_4$), and synthesized AuNPs using the bacterium *Shewanella algae*. XANES spectra showed the oxidation state of synthesized AuNPs using *S. algae* to be Au^{0}, confirming that *S. algae* can reduce Au^{3+} to Au^{0} (Konishi et al., 2007). In this study, MSK03-AuNPs and MSK05-AuNPs were investigated at the Au L3 edge using XANES. As a result, XANES spectra of AuNPs were compared with Au^{0} and Au^{3+}. XANES spectra of MSK03-AuNPs and MSK05-AuNPs represent the region between Au^{0} and Au^{3+}. So, it can be concluded that *S. monashensis* MSK03 and *S. spectabilis* MSK05 cell-free supernatants can reduce $AuCl_4^-$ ions into Au elements.

Medicinal applications of AuNPs depend on the shape, size, and nanoparticle compositions (Hu et al., 2020). The applications of spherical AuNPs of different sizes were shown in Table 5.2. AuNPs are useful for antibacterial effects and attacking intracellular microorganisms due to their small dimensions. It has been reported that the small size of AuNPs demonstrated high antimicrobial activities (Lai et al., 2015; Slavin et al., 2017; Wang et al., 2017). Moreover, the surface chemistry of the synthesized AuNPs is one of the important factors in the properties and functionality of AuNPs. It has been reported the conjugate status of AuNPs for therapeutic and cellular applications. Surface chemistry can be categorized based on the type of surface functionalization, which includes amine, citrate, nucleic acid, lipid, peptide, and antibody ligands. The surface functionalization of amine have been reported an effect on the application of drug delivery, gene transfection, oligonucleotide transfection, and antiviral activity (Giljohann et al., 2010). Several studies have been

reported the characteristic of biosynthesized AuNPs that had chemical stability, well-developed surface chemistry, and appropriate small size easier to interact with bacteria. Antibacterial activity of the biosynthesized AuNPs has been reported against both Gram-negative and Gram-positive bacteria such as *Entercoccus faecium*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *E. coli*, *E. aerogenes*, and *S. aureus.* Moreover, AuNPs can potentially operate as antibiotic transporters or delivery vehicles, increasing the bactericidal activity of antibiotics. AuNPs had the potential to be utilized as antibiotic transporters or delivery vehicles, hence boosting bactericidal action (Bindhu and Umadevi, 2014; Rice, 2010; Shah et al., 2014; Zhang et al., 2015). The possible antimicrobial mechanism of AuNPs has been suggested, such as cell membrane interaction, accumulation on the cell surfaces, and heavy electrostatic attraction (Chamundeeswari et al., 2010; Johnston et al., 2010).

Table 5.2 Medicinal applications of AuNPs with different sizes.

Morphology	Size (nm)	Application/activity	References
Nanosphere	2	Drug delivery	(Peng et al., 2019)
Nanosphere	2-10	Antioxidant	(Tahir et al., 2015)
Nanosphere	4-10	Antimicrobial	(Ahmad et al., 2013)
Nanosphere	5-10	Drug delivery, Bioactivity	(Rossi et al., 2016)
Nanosphere	10-20	Antimicrobial	(Sadhasivam et al., 2012)
Nanosphere	15-20	Antiviral	(Zainab, 2022)
Nanosphere	20-37	Anticancer, Antimicrobial	(Ramalingam et al., 2017)
Nanosphere	100	Therapeutics (PTT and RT)	(Hu et al., 2017)

RT, Radiation therapy; PTT, Photothermal therapy

For the antimicrobial activity of AuNPs that were synthesized using *Streptomyces* sp. Balagurunathan et al. reported antimicrobial activity of biosynthesized AuNPs using *S. viridogens* strain HM10. Spherical AuNPs (18-20 nm particle size) derived from *S. viridogens* HM10 inhibited *S. aureus* and *E. coli* with

inhibition zones of 14 and 20 mm, respectively (Balagurunathan et al., 2011). In this study, the antimicrobial activity against several pathogenic bacteria was measured using the agar well diffusion method. The synthesized AuNPs appear to inhibit the growth of test pathogenies effectively. The inhibition zone of MSK03-AuNPs and MSK05-AuNPs was observed against *S. aureus* TISTR1466*, E. coli* TISTR8465*, P. aeruginosa* N90Ps*, P. aeruginosa* TISTR1287*, A. baumanii, and S. marcescense*. In addition, MSK05-AuNPs also observed inhibition zones against MRSA DMST20651, MRSA DMST20654, and *P. aeruginosa* TISTR781 (Table 4.1).

In most cases, AuNPs have previously been shown ineffective at killing pathogenic bacteria. It has been reported that the bactericidal of biosynthesized AuNPs is very weak at high concentrations (Zhang et al., 2015). The antimicrobial activity of biosynthesized AuNPs using *S. hygroscopicus* BDUS 49 was previously reported by Sadhasivam et al. (2012) Spherical of biosynthesized AuNPs using *S. hygroscopicus* with an average size of 10 to 20 nm inhibited *S. aureus, E. coli*, and *S. typhimurium* with a MIC value of 32 µg/mL. While, AuNPs had MICs of 64, 128, and 256 g/ml against *B. subtilis*, *S. epidermidis*, and *E. faecalis*, respectively (Sadhasivam et al., 2012). Składanowski et al. (2017) reported antimicrobial activity of biosynthesized AuNPs using the supernatant of *Streptomyces sp.* strain NH21. The AuNPs of *Streptomyces* sp. NH21 showed spherical shape with a small size of 10±14 nm. They have been studied the MIC value at various concentrations (1.25–200 µg/mL) of biosynthesized AuNPs using *Streptomyces* sp. NH21 against *Bacillus subtilis*, *E. coli*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* (Składanowski et al., 2017). In this study, the MIC profile of MSK03-AuNPs and MSK05-AuNPs showed MIC values of more than 141.4 µg/mL against tested pathogens.

In conclusion, the *Streptomyces* sp. strains MSK03 and MSK05 were isolated from soil samples. Based on 16S rDNA sequence, the strains MSK03 and MSK05 were identified as *S. monashensis* and *S. spectabilis*, respectively. The extracellular cell-free supernatants of *Streptomyces* sp. MSK03 and MSK05 can be used to synthesize AuNPs. The biosynthesized AuNPs is considered the simple and an eco-friendly process. The biosynthesized AuNPs (MSK03-AuNPs and MSK05-AuNPs) showed spherical shape with particle size ranging from 3.3 to 40 nm. The UV-Vis spectra

confirmed the LSPR band of the biosynthesized AuNPs at 530 and 545 nm. The use of *Streptomyces* sp. for the synthesized AuNPs in the large-scale manufacturing processes has higher growth rates and easier and cheaper cultivation requirements (Gopal et al., 2013; Soltani et al., 2015). Thus, *Streptomyces* sp. MSK03 and MSK05 were found to be a candidate for the AuNPs production.