ENHANCED PRODUCTION OF NITRATE REDUCTASE BY LACTIC ACID BACTERIA FOR THE SYNTHESIZE OF SILVER NANOPARTICLES

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ENHANCED PRODUCTION OF NITRATE REDUCTASE BY LACTIC ACID BACTERIA FOR THE SYNTHESIZE OF SILVER NANOPARTICLES

by

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LIST OF SYMBOLS

℃	Degree Celsius
µ	Micro
%	Percentage
±	Plus-minus Sign
Ag^{+}	Silver Ion
KNO_3	Potassium Nitrate

LIST OF ABBREVIATIONS

AgNPs	Silver Nanoparticles
ATCC	American Type Culture Collection
ANOVA	Analysis Of Variance
ATP	Adenosine Triphosphate
CCD	Central Composite Design
CMC	Carboxymethyl Cellulose
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic Acid
EDX	Energy-Dispersive X-ray spectroscopy
ETN	Erythrityl Tetranitrate
Fe-SEM	Field-emission Scanning Electron Microscopy
ISDN	Isosorbide Dinitrate
ISMN	Isosorbide Mononitrate
LAB	Lactic Acid Bacteria
LSPR	Localized Surface Plasmon Resonance
LFA	Lateral Flow Assay
mM	Millimolar
MEF	Metal-Enhanced Fluorescence
MH	Mueller Hinton
MRS	de Man, Rogosa and Sharpe
MRSA	Methicillin-resistant *Staphylococcus aureus*

mV	Millivolt
nm	Nanometer
NAS	Assimilatory Nitrate Reductases
NAR	Respiratory Nitrate Reductases
NAP	Periplasmic Nitrate Reductases
NTG	Nitroglycerin
pH	Potential of Hydrogen
PDI	Polydispersity Index
PETN	Pentaerythrityl Tetranitrate
PE	Polyethylene
PLA	Polylactic Acid
PVC	Polyvinyl Chloride
RNA	Ribonucleic Acid
rpm	Revolution Per Minute
ROS	Reactive Oxygen Species
RSM	Response Surface Methodology
SPR	Surface Plasmon Resonance
SERS	Surface-Enhanced Raman Scattering
UV-Vis	Ultraviolet-Visible spectroscopy

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PENINGKATAN PENGHASILAN NITRAT REDUCTASE OLEH BAKTERIA ACID LAKTIK BAGI SINTESIS NANOPARTIKEL PERAK

ABSTRAK

Di antara nanoteknologi yang baru muncul, nanozarah perak mendapat banyak perhatian kerana fizikokimianya yang unik. Kajian ini bertujuan untuk mengoptimumkan pengeluaran nitrat reduktase dalam bakteria asid laktik untuk peningkatan pengeluaran nanozarah perak. Pencilan bakteria asid laktik disaring untuk kemampuan pengurangan nitrat dan aktivitinya diukur menggunakan ujian enzimatik nitrat reduktase. Selepas saringan, *Lactobacillus plantarum* CAM 4 telah dipilih untuk pengoptimuman aktiviti nitrat reduktase. Metodologi permukaan respons digunakan untuk mengoptimumkan medium pengeluaran enzim. Pemboleh ubah yang terlibat dalam kajian ini adalah glukosa, kalium nitrat (KNO_3), dan pH. Medium yang telah dioptimumkan mengandungi 0.75% glukosa, 2% KNO_3, dan pH 5.0 dapat menghasilkan aktiviti nitrat reduktase sebanyak 121.77 U / mL. Dalam kajian pengoptimuman, aktiviti nitrat reduktase berjaya meningkat 53.56% dari 79.30 U / mL menjadi 121.77 U / mL setelah pengoptimuman. Nanozarah perak disintesis telah dicirikan menggunakan spektroskopi UV-Vis, penyebaran cahaya dinamik (DLS), potensi zeta, mikroskop elektron penghantaran (TEM), mikroskop elektron pengimbasan pelepasan medan (FE-SEM) dan analisis sinar-X penyebaran tenaga (EDX). Analisis spektrofotometri menunjukkan bahawa nanozarah perak biosintesis menunjukkan puncak pada 430 nm. Analisis DLS menunjukkan ciri nanozarah perak yang tersebar. Analisis TEM menunjukkan bahawa nanozarah perak berbentuk bulat dengan ukuran purata 19.2 ± 4.8 nm. FE-SEM mendedahkan morfologi permukaan

nanozarah perak biosintesis, sementara spektrum analisis EDX mendedahkan komposisi unsur nanozarah perak. Lebih-lebih lagi, nanozarah perak biosintesis menunjukkan aktiviti antibakteria terhadap patogen Gram-positif dan Gram-negatif. Hasil dari kajian ini memberikan pandangan penting mengenai kaedah lestari untuk peningkatan pengeluaran nanozarah perak menggunakan bakteria asid laktik.

ENHANCED PRODUCTION OF NITRATE REDUCTASE BY LACTIC ACID BACTERIA FOR THE SYNTHESIZE OF SILVER NANOPARTICLES

ABSTRACT

Among the emerging nanotechnology, silver nanoparticles (AgNPs) get much attention due to their unique physicochemical. The present study aimed to optimize nitrate reductase production in lactic acid bacteria (LAB) for enhanced production of AgNPs. LAB isolates were screened for nitrate reduction ability and their activities were quantified using nitrate reductase enzyme assay. *Lactobacillus plantarum* CAM 4 was selected for the optimization of nitrate reductase activity due to highest nitrate reductase activity measured among LAB isolates. Response surface methodology was employed to optimize the medium to produce nitrate reductase. The variables involved in this study were glucose, potassium nitrate (KNO_3), and pH. The optimized medium containing 0.75 % of glucose, 2 % of KNO_3, and pH 5.0 resulted in a nitrate reductase activity of 121.77 U/mL. In the optimization study, the nitrate reductase activity was successfully increased 53.56% from 79.30 U/mL to 121.77 U/mL after optimization. The biosynthesized of AgNPs were characterized using UV-visible spectroscopy (UV-Vis), dynamic light scattering (DLS), zeta potential, transmission electron microscope (TEM), field-emission scanning electron microscope (FE-SEM) and energy dispersive X-ray (EDX) analysis. Spectrophotometric analysis revealed that the biosynthesized AgNPs exhibited a peak at 430 nm. DLS analysis indicated the monodispersed characteristic of AgNPs. TEM analysis revealed that the AgNPs were spherical with average size of 19.2 ± 4.8 nm. FE-SEM revealed revealed the surface morphology of biosynthesized AgNPs, while EDX analysis spectrum revealed the

elemental composition of AgNPs. Moreover, biosynthesized AgNPs exhibited antibacterial activity against Gram-positive and Gram-negative pathogens. Results from this study provide notable insights on sustainable method for the enhanced production of AgNPs using LAB.

CHAPTER 1

INTRODUCTION

1.1 Research background

Nanotechnology is defined as the science and engineering associated with the design, synthesis, characterization, and usage of nanometer-scale materials and devices (Silva, 2004). At these scale, individual molecules and groups of molecules that interact must be taken into account in relation to the overall macroscopic properties of the material or device. This is because changing the basic molecular structure makes it possible to change the chemical and physical properties at the macroscopic scale (Khan et al., 2019). Recently, the field of nanotechnology has experienced rapid growth and significant advancements.

Due to the wide range of applications that nanoparticles have, researchers have shown a great deal of interest in them. In the last few decades, one of the most researched types of nanoparticles are silver nanoparticles (AgNPs) (Zhang et al., 2016). Silver nanoparticles (AgNPs) are defined as nanoparticles of silver, which are generally between 1 and 100 nm in size. Using the citrate reduction procedure, M.C. Lea made the initial discovery and recording of AgNPs in 1889 (Nowack et al., 2011). Gradually, many different methods for synthesizing AgNPs have been discovered and utilized. Generally, two main approaches are used for synthesizing AgNPs: top-down and bottom-up approaches. The top-down approach entails breaking down the bulk material into nanosized structures or particles in order to create the nanoparticles from larger entities. In contrast, in the bottom-up approach, the nanoparticles are constructed from molecular

building blocks that chemically assemble themselves using the molecular recognition principle (Lee & Jun, 2019). Nowadays, these approaches for AgNPs synthesis are further categorized into physical, chemical and biological methods.

Physical methods manufacture AgNPs with a narrow size distribution by utilizing physical energies. A vast quantity of AgNPs can be produced in a process by using physical methods. Evaporation-condensation and laser ablation are two instances of physical methods. In the evaporation-condensation method, atmosphere-pressured tube furnace is filled with the source material. The source material is then evaporated using high temperature, which leads to the formation of nanoparticles (Iravani et al., 2014). As for the laser ablation method, a laser beam is used to illuminate a plate containing silver in a liquid medium. A hot plasma containing AgNPs is created when the laser beam is absorbed by the metal plate. The synthesis of AgNPs is initiated when the temperature is lowered by the surrounding liquid medium (Pyatenko et al., 2004). The primary advantage of both physical approaches is the absence of reducing and stabilising agents; hence, the generated AgNPs are contaminant-free and do not require further purification. The major drawback of physical methods is that they are energy-intensive and require a long time to reach a stabilised temperature to initiate the synthesis of AgNPs (Singh & Kaur, 2020).

The AgNPs are mostly produced via chemical processes. Silver ions are converted into silver atoms via these chemical processes, and the oligomeric clusters that result from these processes give rise to AgNPs. These methods commonly used silver nitrate as the precursor. The reducing agents can be categorized into strong and weak reducing agents. Strong reducing agents are able to produce large-sized monodispersed nanoparticles, whereas weak reducing agents can produce nanoparticles with wide size distribution.

Various reducing agents such as borohydride, citrate, ascorbic acid, hydrazine, and ethylene glycol are used to reduce the silver ions which leads to the formation of AgNPs. Other than that, the surface morphology of AgNPs is also affected by the type of dispersion medium (Natsuki, 2015). Chemical methods have the advantages of high yield and ease of production but involve the use of chemical agents that are toxic, which then produce hazardous byproducts and waste. Besides that, AgNPs produced from these methods have the problem of chemical contamination (Ganaie et al., 2015).

To overcome these limitations, biological methods are employed nowadays by using natural reducing agents to produce AgNPs. Biological methods mainly involve the use of microorganisms (bacteria, fungi, and yeasts) and plant. Plants and microorganisms contain an abundance of natural reducing agents that can reduce silver ions into AgNPs, such as enzymes, carbohydrates, and different compounds in plant extracts. Besides that, there is also the availability of natural stabilizing agents such as secondary metabolites, amino acids, and proteins (Srikar et al., 2016). Bacteria are widely explored and utilized in the synthesis of AgNPs due to the fact that they are consistent and a good choice as a nanofactory for producing different types of nanoparticles (Xu et al., 2018). Previous studies have discovered that nitrate reductase, an enzyme found in both eukaryotes and prokaryotes, plays a major role in the biosynthesis of AgNPs. An electron shuttle is induced when nitrate reductase converts nitrate into nitrite, which reduces the incoming silver ions to AgNPs (Mukherjee et al., 2018). In recent years, many studies have been done on how lactic acid bacteria (LAB), which are often known as probiotic bacteria, can be used to synthesize AgNPs. Several studies have demonstrated that the cell biomass and the supernatant of LAB can be used as reducing agent for the synthesis of AgNPs (Mohd

Yusof et al., 2020; Rajesh et al., 2015). However, the study on the influence as well as optimization of nitrate reductase activity on the biosynthesis of AgNPs using LAB is scant.

1.2 Problem statement

There are various drawbacks to the synthesis of AgNPs using physical and chemical methods. The primary disadvantage of physical approaches is that they require a lot of energy and take time to stabilise the temperature needed to start the synthesis of AgNPs. Chemical methods involve the use and production of chemical reducing agents and byproducts that are toxic, respectively. Besides, the AgNPs synthesized by chemical methods have the issue of chemical contamination. Biological synthesis of AgNPs by microorganisms, particularly LAB, has been a great alternative to conventional methods. However, the study on the influence as well as optimization of nitrate reductase activity on AgNPs synthesized from LAB is lacking.

1.3 Research scope and objectives

This study focuses on optimization of nitrate reductase production in LAB to enhance the production of AgNPs, as well as to evaluate the antimicrobial activities of biosynthesized AgNPs against pathogenic bacteria.

Objectives

i. To screen the locally isolated lactic acid bacteria with highest nitrate reductase activity.
ii. To optimize the parameters for the production of nitrate reductase by selected lactic acid bacteria (LAB) using response surface methodology (RSM).
iii. To characterize the silver nanoparticles (AgNPs) synthesized using selected lactic acid bacteria (LAB) and evaluate the antimicrobial activities against pathogenic bacteria.

CHAPTER 2

LITERATURE REVIEW

2.1 Lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria, non-respiratory, non-spore forming, cocci, coccobacilli or rods, which produce lactic acid as major end product. LAB are well known for their significant role in a variety of fermentation processes, involving in food, agricultural, and clinical applications. LAB are generally recognised as safe (GRAS) due to their wide range of applications, except for a few species in the genera *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Carnobacterium* (Bintsis, 2018). LAB can be found in many places, such as plants and fermented foods, as well as in soil, water, and the digestive tracts of animals. LAB were grouped according to their morphology, mechanism of glucose fermentation, capacity for growing at various temperatures, and utilisation of sugars as carbon sources. Due to their GRAS status, LAB are widely studied and utilised as microbial cell factories. It is undeniable that the contemporary uses of LAB go beyond the conventional food fermentation processes to include the use of LAB as medicine delivery systems, as vaccines, and as microbial cell factories for industrially relevant metabolites (Mozzi, 2016).

2.2 Nitrate-reducing bacteria

Nitrate-reducing bacteria (NRB) refers to a group of bacteria that have the ability to reduce nitrate to nitrite or other nitrogenous compounds. Some common examples of active nitrate reducers that can be found in the nature, such as in waters enriched with

organic matters and nitrate, are *Achromobacter, Bacillus*, *Corynebacterium*, *Micrococcus denitrificans*, *Pseudomonas*, *Serratia*, and *Vibrio* species (Shukla et al., 2021). The majority of nitrate reducing bacteria are facultative anaerobes and therefore able to alternate between oxygen and nitrate respiration based on the surrounding environment (Luque-Almagro et al., 2011). Nitrate-reducing oral bacteria are also found in human oral cavity, for examples, *Haemophilus*, *Neisseria, Prevotella*, *Streptococcus*, and *Veillonella* are the most frequently identified nitrate reducing bacteria (Hyde et al., 2014). Besides, other studies also have shown that human gut contains nitrate reducing bacteria, suggesting that some gut bacteria use nitrate as a nutrient, as well as a final electron acceptor during respiration (Kraft et al., 2011; Rocha & Laranjinha, 2020). Tiso and Schechter (2015) demonstrated that several species of lactic acid bacteria from human gut were able to reduce nitrate to nitrite or other nitrogenous compounds (Tiso & Schechter, 2015).

2.3 Nitrate reductase

Nitrate reductase belongs to a group of enzymes containing molybdenum which is responsible for reducing nitrate (NO3-) to nitrite (NO2-), as well as for catalyzing two-electron transfer reactions in carbon, nitrogen, and sulphur cycles (Kisker et al., 1997). Nitrate reductase can be categorized into two types: eukaryotic nitrate reductase and prokaryotic nitrate reductase. Only nitrate assimilation is carried out by eukaryotic nitrate reductases, which are found in plants and fungi. Meanwhile, both assimilatory and dissimilatory nitrate reduction can be carried out by prokaryotic nitrate reductases. There are three distinct classes of nitrate reductase in prokaryotes: assimilatory nitrate reductases

(NAS), respiratory nitrate reductases (NAR), and periplasmic nitrate reductases (NAP) (Stolz & Basu, 2002). All nitrate reductases have been classified according to their subcellular location, structure of molybdenum active site, and physiological function. Assimilatory nitrate reductases (NAS) are engaged in the assimilation of nitrogen and situated in the cytoplasmic compartment. Respiratory nitrate reductases (NAR) are located on cytoplasm membrane and actively engaged in anaerobic nitrate respiration. Periplasmic nitrate reductases (NAP) are located in periplasmic compartment and involved in nitrate dissimilation (Richardson et al., 2001). During assimilatory nitrate reduction, the biomass of the organism incorporates nitrogen derived from nitrate. The nitrate is reduced to nitrite, and then nitrite is converted into ammonia to be used by eukaryotes. In contrast, dissimilatory nitrate reductase excretes the end products from the cell instead of incorporating nitrogen into the biomass (Sparacino-Watkins et al., 2014).

2.4 Nitrate

Nitrate can be categorized into two different forms: inorganic and organic nitrate, based on their chemical structure (Holland & Weitz, 2003). The most prevalent form of inorganic nitrate is nitric acid salt, which has a negatively charged ion made up of one nitrogen atom and three oxygen atoms. The positively charged regions of polar water molecules can interact with inorganic nitrates, making them soluble in water. Inorganic nitrate salts can occur naturally through the fixation of atmospheric nitrogen and oxygen as a part of nitrogen cycle in the environment (Holland & Weitz, 2003; Laue et al., 2000). Inorganic nitrate salts have a wide range of applications due to their high solubility, oxidizing properties, and the presence of freely available nitrogen. For examples, nitrates

are mainly used as the fertilizers in agriculture as freely available nitrogen ions can be easily absorbed by the plant for growth, aided by the high solubility of nitrates. Besides, due to their strong oxidizing properties, inorganic nitrate salts such as potassium nitrate and sodium nitrate are widely used in explosives. Inorganic nitrates also served as food additives which act as colour retention agent and preservative in cured and processed meats.

The organic forms of nitrates are more complex compared to inorganic nitrates, as they are synthetic compounds, the esters are produced from the reaction between nitric acid and an alcohol group, the reaction is known as nitro-oxylation (J. Liu, 2019; Omar et al., 2012). For over a century, organic nitrates have been used in medicine as potent vasodilators to treat cardiovascular diseases such as angina pectoris, acute coronary syndrome, and heart failure (Daiber & Münzel, 2015; França-Silva et al., 2014). The main types of organic nitrates used in medicine are: nitroglycerin (NTG), isosorbide dinitrate (ISDN), isosorbide mononitrate (ISMN), pentaerythrityl tetranitrate (PETN), and erythrityl tetranitrate (ETN). Other than that, organic nitrates have also been studied to be potential supplementation on improving human muscle power (Coggan et al., 2021), as well as to treat osteoporosis, therefore reducing the risk of morbidity, mortality, and costs associated with osteoporotic fractures (Bucur et al., 2013).

2.5 Synthesis of AgNPs by bacteria

One of the most promising choices for nanoparticle manufacturing is bacteria due to their extraordinary capacity to reduce heavy metal ions. To counteract pressures such as the toxicity of heavy metals, for example, some bacterial species have adapted to use

defence mechanisms. Some of them were shown to be able to withstand and grow even in environments with high metal ion concentrations (Iravani, 2014). As a result, many bacterial species are being studied for this purpose (Singh et al., 2018).

Table 2.1 Some bacterial species previously studied for the biosynthesis of AgNPs.

Bacterial species	Route	Size (nm)	Reference
Penicillium glabrum	Extracellular	26-32	(Nanda and Majeed, 2014)
Serratia nematodiphila	Extracellular	10-31	(Malarkodi et al., 2013)
Lactobacillus casei subsp. casei	-	25-50	(Korbekandi et al., 2012)
Novosphingobium sp. HG-C3	Extracellular	8-25	(Du et al., 2016)
Lactobacillus spp.	Extracellular and intracellular	2-20	(Ranganath et al., 2012)
Escherichia coli	Extracellular	10-100	(Ghorbani, 2013)
Bacillus subtilis	Extracellular	5-50	(Saifuddin et al., 2009)
Bacillus sp.	Intracellular	5-15	(Pugazhent et al., 2009)
Actinobacteria	Intracellular	5-50	(Suman et al., 2014)
Lactobacillus sp.	Extracellular	30-100	(Dakhil, 2017)
Lactobacillus acidophilus	Intracellular	30	(Garmasheva et al., 2016)
Lactobacillus plantarum	Intracellular	14	(Mohd Yusof et al., 2020)
Lactobacillus plantarum	Extracellular	33.4	(Garmasheva et al., 2016)
Lactobacillus sp.	Extracellular	14	(Matei et al., 2020)

2.6 Mode of action of AgNPs against pathogenic bacteria

A wide range of microorganisms have been shown to be inhibited by AgNPs. The exact mechanism behind their manner of antibacterial action, however, is still not well understood (Malarkodi et al., 2014). Even so, it has been established that AgNPs exhibit some fundamental mode of action against pathogenic bacteria. AgNPs' mode of action is correlated with four distinct pathways: interaction with cell walls and membranes, penetration into the cell followed by disruption of intracellular components, induction of

oxidative stress and cellular toxicity, and modulation of signal transduction pathways (Dakal et al., 2016).

When bacteria are exposed to AgNPs, the AgNPs can adhere to the surface of the bacteria. It was revealed that the surface charge of AgNPs can affect how they adhere and initiate interaction with bacterial cell wall and membrane. For instance, a positively-charged AgNPs can bind to negatively-charged bacterial cell membrane effectively due to electrostatic attraction (Abbaszadegan et al., 2015). It was demonstrated that such adhesion results in cell wall and membrane disruption, leading to cytoplasmic shrinkage (McQuillan et al., 2012). Previous studies reported that the cell wall and cell membrane of *E. coli* were disrupted by AgNPs when observed using transmission electron microscopy (TEM) (Sondi & Salopek-Sondi, 2004). Other than that, AgNPs can also interact with proteins in the cell wall that contain sulfur. This changes the structure and permeability of the cell membrane, which interrupts the normal transport activity of the cell (Ghosh et al., 2012). For example, silver ions released by AgNPs can impair the uptake and release of potassium ions in bacteria (Nishihara et al., 2022). Other than that, an alteration in cell permeability can directly cause the leakage of cellular components (J. Li et al., 2013). Moreover, AgNPs can also cause genetic alternations in bacterial cells, triggering cell apoptosis (Rai et al., 2012).

AgNPs have the ability to penetrate bacterial cells and alter critical biological processes (Singh et al., 2015). Silver ions released by AgNPs can interact with deoxyribonucleic acid (DNA), enzymes, and lipids when they invade a bacterial cell. Previous studies revealed that protein synthesis in bacterial cells was inhibited due to the interaction of AgNPs with ribosomes (Jung et al., 2008; Morones et al., 2005). It has been

demonstrated that silver ions may bind with the proteins' functional groups, deactivating the proteins as a result. For instance, protein inactivation occurs when silver ions form stable SAAg interactions with the thiol groups of proteins present in the cell membrane (Klueh et al., 2000; Rai et al., 2012).

Other than that, AgNPs can also inhibit the sugar metabolism in bacterial cells. According to a report, the interaction of phosphomannose isomerase with AgNPs results in its deactivation, which inhibits the metabolism of sugar (Bhattacharya & Mukherjee, 2008). Additionally, the interaction of AgNPs with genetic material may result in denaturation and a halt to cell division (Hsueh et al., 2015). It has been found that silver ions combine with nucleic acids to make complexes where they interact with nucleosides. The silver ion intercalates between the purine and pyrimidine base pairs, breaking the hydrogen bonds and the DNA structure (Klueh et al., 2000). Additionally, AgNPs also changes the state of the DNA molecule from relaxation to condensation, which impairs its capability for replication (Feng et al., 2000).

AgNPs can also make reactive oxygen species (ROS) and free radical species, which are harmful to cells and cause oxidative stress (Wu et al., 2014). It was reported that the adhesion of silver ions to the bacterial cell membrane causes cellular toxicity by blocking mitochondrial normal function (Blecher Paz & Friedman, 2012). The mitochondrial membrane is also directly harmed by an excess of free radicals that are produced, inducing cell apoptosis (Zorov et al., 2014). It was discovered that AgNPs' interactions with cell membranes and the production of ROS had a synergistic effect on *Pseudomonas aeruginosa* (Yan et al., 2018). The fatty acids in the membrane can be oxidised by ROS, which leads to the production of more free radicals and harm to the cell

membrane (Rajeshkumar & Bharath, 2017). The generation of free radicals is well known to react and break the components of DNA (Pilger & Rüdiger, 2006; Valavanidis et al., 2009).

It is well known that bacteria phosphorylate a variety of protein substrates (Deutscher & Saier Jr., 2005). In bacteria, the process of phosphorylation cascade is a signal relay process that is crucial for growth and cellular function (Kirstein & Turgay, 2005). When bacteria are exposed to AgNPs, cellular signalling can be modified by AgNPs, which can dephosphorylate the building blocks of protein (Shrivastava et al., 2007). The building blocks of proteins, also known as phosphorylated proteins, are important in basic cellular functions such as DNA replication. Therefore, dephosphorylation of these proteins can essentially disrupt normal cellular growth (Garcia-Garcia et al., 2016). Previous studies have demonstrated dephosphorylation of proteins in *E. coli* and *Salmonella typhi* when bacteria are exposed to AgNPs, indicating that the bacterial growth was inhibited (Shrivastava et al., 2007).

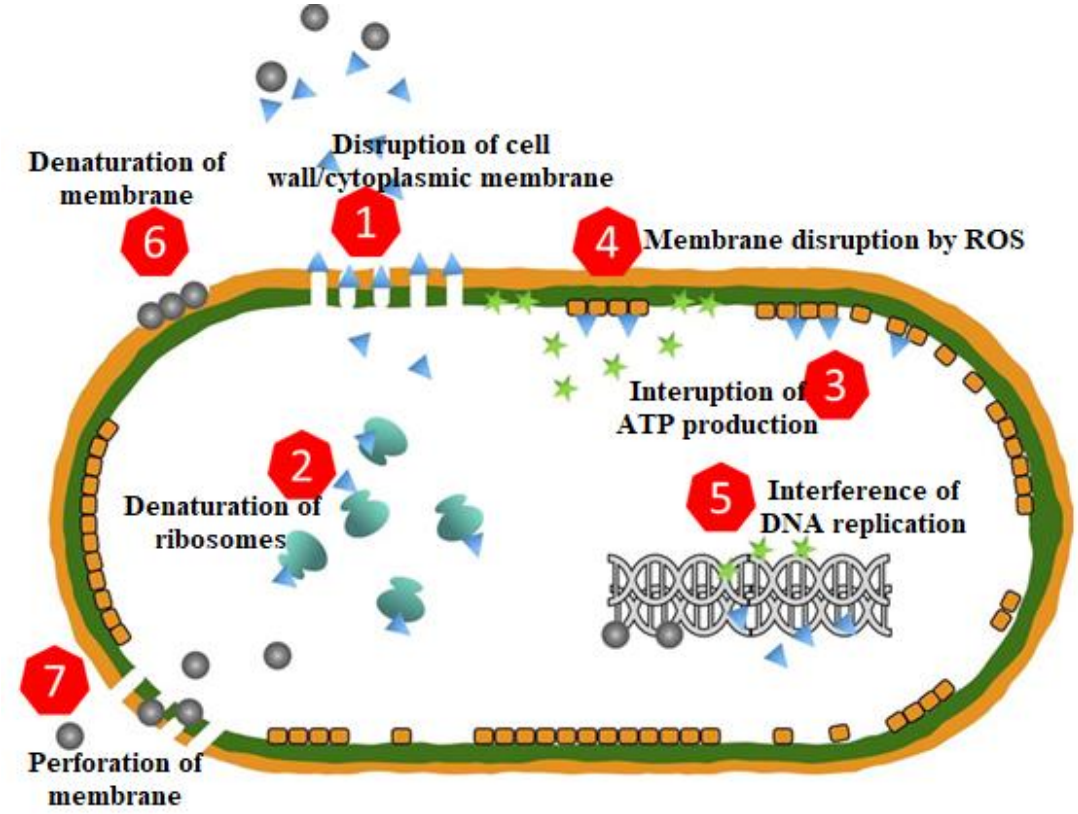

Figure 2.1 The antibacterial mechanism of AgNPs. Extracted from Yin (2020).

2.7 Applications of silver nanoparticles (AgNPs)

Due to their unique physical and chemical properties, AgNPs have been widely utilized and increasingly used in various fields, including conductive, diagnostic, optical, food industry, consumer product, agriculture, and medical applications.

2.7.1 Conductive

In the field of flexible electronics, inkjet printing has been looked into as an alternative production tool for the fabrication of conductive parts and devices. The printed design is transformed into conductive elements using this fabrication method, which involves depositing particles of the material with the desired electrical characteristics onto a substrate (Fernandes et al., 2020). Among other elements, silver continues to be one of

the best choices for use as conductive ink. This is mostly because of its excellent thermal and electrical conductivity, chemical stability, affordability, and electrical conductivity in its oxide form (Ren et al., 2015). AgNPs used for conductive ink are commonly synthesized by chemical reduction method, using silver nitrate as the precursor and various organic and inorganic reducing agents (Cao et al., 2017).

Additionally, AgNPs can be also used in electronic paste due to their excellent conductivity. Electronic paste is a type of conductive adhesive that is primarily used as a glue for electronics. A variety of conductive components such as gold, silver, copper, and aluminum are used in electronic paste (Li et al., 2020). Among these conductive components, gold has the best conductivity but requires relatively high cost. Copper and aluminum are cheaper than gold and have excellent conductivity, but they are easily oxidized which reduced the conductivity. At present, silver is the most preferred conductive component due to its excellent stability, conductivity, and lower cost (Richner et al., 2016; Tomotoshi & Kawasaki, 2020; Y. Wang et al., 2019).

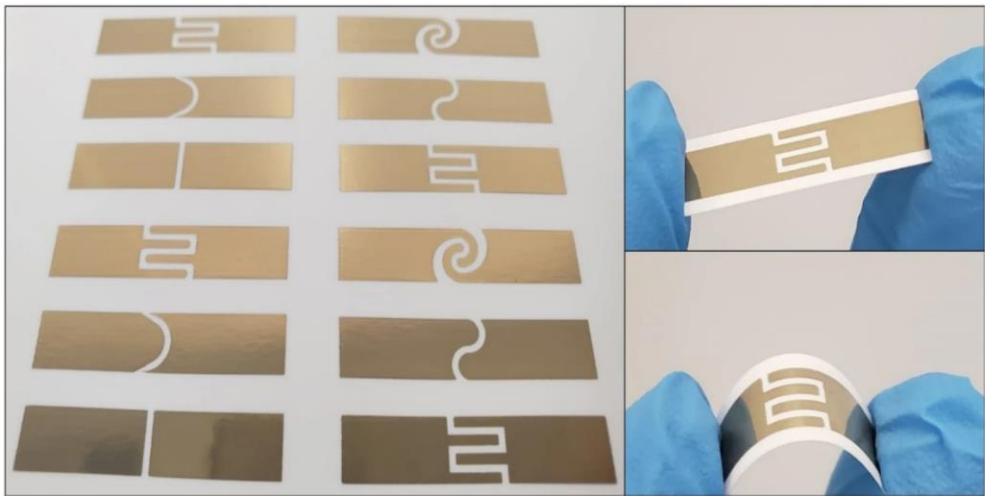

Figure 2.2 Printed electrodes with various designs made from conductive inks containing AgNPs. Extracted from Fernandes (2020).

2.7.2 Diagnostic

AgNPs have unique surface plasmon resonance (SPR) properties, which makes them absorb and scatter light efficiently. SPR refers to a collective oscillation that occurs when conduction electrons on the metal surface are excited by light at a specific wavelength (Lee & Jun, 2019). Therefore, AgNPs are suitable to be used diagnostic applications such as biosensors, assays, and quantitative detection (Bollella et al., 2017). Previous studies have demonstrated that AgNPs can be utilized as biosensors to detect variety of components, such as molecular markers, proteins, glucose, etc. For instance, AgNPs were used by researchers as a conductive addition to create a highly sensitive biosensor for the detection of glucose. The interaction sites between AgNPs and glucose were improved by both the porous AgNPs nanostructures and the high surface areas of the carriers. This might hasten AgNPs' electron transfer and boost the biosensor's sensitivity (Anderson et al., 2017).

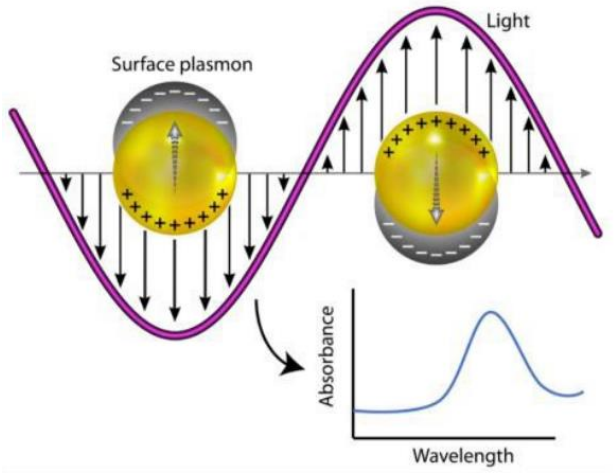

Figure 2.3 Localized surface plasmon resonance (LSPR) of AgNPs, which results in a unique absorption peak. Extracted from Masson (2020).

AgNPs have also been used to identify the presence of p53 protein in cancer cells. p53 protein present in humans is encoded by the TP53 gene and its crucial function as a tumour suppressor protein is widely understood. Point mutations in the TP53 gene can overly increase the production of the p53 protein when tumours form, which promotes uncontrolled cell division and the development of cancer. AgNPs-based localised surface plasmon resonance (LSPR) nanosensor was created to detect p53 in vitro, offering a viable platform for molecular tumour diagnostics (Zhou et al., 2011).

Additionally, AgNPs have the potential to be effective as fluorescent markers for bioassays. By integrating the rapid separation of magnetic beads and AgNPs labelled antibodies, an AgNPs-enhanced immunofluorescence assay for the detection of biomarkers was developed (Kurdekar et al., 2017). Besides, the development of a multiplexed AgNPs-based lateral flow assay (LFA) that can distinguish between different pathogens has also made it possible to conduct successful diagnostic studies (Yen et al., 2015).

2.7.3 Optical

AgNPs are frequently utilised as probes for metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS). AgNPs demonstrate more probing benefits than other noble metal nanoparticles, including greater extinction coefficients, high field enhancements, and sharper extinction bands (Caro et al., 2010). MEF is the interaction between the excited state of the fluorophore and the localised surface plasmons of metallic nanoparticles, which enhances the radiative quantum yield and improves fluorescence (Aslan et al., 2008). The detection of small protein can be accomplished using AgNPs and fluorophore in MEF (Duchesne & Fernig, 2007).

By utilizing an enhanced electromagnetic field on the surface of AgNPs, AgNPs can be used as nanoscale antennas in Raman spectroscopy to amplify the Raman signals (Lee & Jun, 2019). Adsorption of molecules onto AgNPs aggregates with plasmonic nanostructures is necessary for SERS detection (Botta et al., 2013). The SERS effect is the interaction between the vibrational states of the molecules that have been adsorbed and the surface plasmons of the metal, and this effect can be used to detect specific proteins or biomolecules effectively (Wurtz et al., 2003).

2.7.4 Food industry

The antimicrobial properties of AgNPs against bacteria, viruses, and fungi are well recognised (Dakal et al., 2016). Recently, AgNPs have been studied and used as an antimicrobial agent in food packaging technologies because both consumers and food processors demand safe and high-quality foods (He & Hwang, 2016). Antimicrobial food packaging has the potential to release biocide agents that are active, enhancing food quality, increasing shelf life, and avoiding spoiling (Istiqola & Syafiuddin, 2020). Several studies have found that polymers such as dextran and chitosan can be utilised as a medium for AgNPs synthesis to produce nanocomposite films for antimicrobial food packaging (Kraśniewska et al., 2020).

Table 2.2 Various studies on packaging materials incorporated with AgNPs.

Food packaging material	Main finding	Reference
Polylactic acid (PLA), AgNPs, nanoclay	The films exhibited enhanced water barrier and antibacterial activity	(Busolo et al., 2010)
Corn starch, AgNPs	The films exhibited antimicrobial activity and enhanced films properties	(Yoksan & Chirachanchai, 2010)
Polylactic acid (PLA), AgNPs, nanocrystalline cellulose	The films exhibited antibacterial activity against Gram-positive and Gram-negative bacteria	(Fortunati et al., 2012)
Agar, AgNPs	There was enhancement of gas and water barrier, tensile properties	(Rhim et al., 2013)
Polylactic acid (PLA), AgNPs, cellulose	Enhanced barrier properties	(Fortunati et al., 2014)
Gelatin, AgNPs, nanoclay	Strong antimicrobial activity against foodborne pathogens	(Kanmani & Rhim, 2014)
Pullulan, essential oils, AgNPs	Edible films for food packaging	(Morsy et al., 2014)
Carboxymethyl cellulose (CMC), AgNPs	Strong antibacterial activity against Gram-positive and Gram-negative bacteria	(Nile et al., 2020)
Agar, banana, AgNPs	Strong antimicrobial properties	(Orsuwan et al., 2016)
Polyvinyl chloride (PVC), AgNPs	The films exhibited antibacterial and antifungal properties	(Shimoga et al., 2019)
Polyethylene (PE), AgNPs	Enhanced antimicrobial properties	(Becaro et al., 2015)

Additionally, AgNPs also have been utilised in water and wastewater treatment. In this area, major contaminants including pesticides, heavy metals, and microbes are primarily removed using AgNPs (Que et al., 2018). Previous studies have employed AgNPs in membrane filters for water purification (Lin et al., 2013). AgNPs applied to filter materials have been considered a promising method to disinfect water due to their significant antimicrobial activity (Quang et al., 2013). For instance, a blotting paper sheet with AgNPs attached to the cellulose fibres shown significant antimicrobial activity against pathogens during filtration (Dankovich & Gray, 2011).

2.7.5 Consumer products

Due to their excellent antimicrobial properties, numerous consumer products frequently contain AgNPs as a component. For example, AgNPs have been used as an ingredient in cosmeceuticals to enhance appearance (Mukta & Adam, 2010). Particularly during the manufacturing or storage phases, cosmetic goods are susceptible to germs and fungi. Since cosmetics come into contact with human bodies directly, it's critical to keep pathogens out of them (Pulit-Prociak & Banach, 2016). Additionally, AgNPs are also used as an ingredient in soap products. Previous studies also mentioned that AgNPs possess antimicrobial and anti-inflammatory effects in soap products (Ong & Nyam, 2022). Moreover, AgNPs have been also incorporated onto textile to exhibit antimicrobial properties. Previous studies demonstrated that AgNPs incorporated onto cotton fabric shown effective antibacterial activity (Gokarneshan, 2017; Zhang et al., 2009). Other than that, AgNPs are commonly found as an active ingredient in socks to inhibit bacteria growth and prevent bad smells (Mohan et al., 2019).

2.7.6 Agriculture

By improving seed germination and plant growth, AgNPs have been considered as a possible contender to boost crop productivity. Previous studies demonstrated that a specific concentration of AgNPs is needed for different plants to achieve maximum growth enhancement, as an incorrect concentration could have a negative effect on plant growth. The use of AgNPs could significantly improve seed germination potential, index, and mean time(Kale et al., 2021). With the use of AgNPs, researchers have developed a notable breakthrough in plant disease control. There is evidence that AgNPs work against pathogens that cause plant diseases, showing antibacterial and antifungal properties (Alloway, 2008; Polash et al., 2017). Nowadays, pests are posing a greater threat to the agricultural industry, affecting crop productivity and therefore the quality of the harvests. Researchers have found that AgNPs can be used as a non-toxic, safe, and effective approach to combat pests (Anand & Bhagat, 2019).

2.7.7 Medical

2.7.7(a) Antibacterial

Antibiotic resistance in pathogenic bacteria may be overcome by AgNPs, which appear to be alternative antimicrobial agents. Therefore, a variety of nanomaterials have been widely studied to overcome antibiotic resistance in pathogenic bacteria. AgNPs appear to be viable antibacterial agents among the many promising nanomaterials because of their unique properties. Previous studies demonstrated that AgNPs showed excellent antibacterial activity against *Escherichia coli*. It was reported that AgNPs accumulated in the bacterial cell wall, causing membrane denaturation that led to cell death (Sondi & Salopek-Sondi, 2004). It was reported that the antibacterial activity of AgNPs depends on

their size. Smaller AgNPs have a larger surface-to-volume ratio to interact with the target bacteria, therefore showing greater antibacterial activity than larger AgNPs (Baker et al., 2005). Additionally, the antibacterial action of AgNPs is influenced by their shape. Among various shapes, spherical-shaped AgNPs have been found to have a stronger antibacterial activity due to larger surface-to-volume ratio as mentioned previously (Hong et al., 2016). Due to their multi-targeting mechanisms, AgNPs have an antibacterial activity that is unaffected by mechanisms of antibiotic resistance (Rai et al., 2012). Additionally, AgNPs have demonstrated a promising synergy with traditional antibiotics in the fight against multi-resistant bacteria (Baptista et al., 2018).

Table 2.3 Previous studies on synergistic effect between AgNPs and antibiotics.

Antibiotics combined with AgNPs	**Pathogen tested**	**Main findings**	**Reference**
Ampicillin	*Escherichia coli*, Ampicillin-resistant *E. coli*, *Klebsiella pneumoniae*, Ampicillin-resistant *Staphylococcus aureus*, MRSA	Enhanced antibacterial activity was observed against all pathogens, AgNPs combined with ampicillin reduced the CFU even in resistant strains	(Brown et al., 2012; Khatoon et al., 2019)
Azlocillin	*Pseudomonas aeruginosa*	Enhanced antibacterial activity	(Alizadeh et al., 2017)
Vancomycin, ampicillin, penicillin	*S. aureus*, *E. coli*, *K. pneumoniae*	Increased antibacterial activity when combined with AgNPs	(Ashmore et al., 2018)
Amikacin, vancomycin	*E. coli*, *S. aureus*	Improved inhibition zone was observed, amikacin have better synergistic effect with AgNPs compared to vancomycin.	(Kaur & Kumar, 2019)
Aztreonam, ampicillin, biapenem, kanamycin, Chloramphenicol,	*E. coli*, *Salmonella typhymurium* *S. aureus*, *Bacillus subtilis*	Kanamycin exhibited the best synergistic effect with AgNPs among the antibiotics	(Vazquez-Muñoz et al., 2019)

2.7.7(b) Antifungal and antiviral

Immunosuppressed people are more likely to get fungal infections, and treating diseases caused by fungi can be difficult (Kim et al., 2008). Many studies have demonstrated AgNPs as a promising antifungal agent. AgNPs exhibited excellent antifungal activity against a variety of pathogenic fungi, such as *Candida albican*, *Candida glabrata*, *Aspergillus niger*, *Cryptococcus neoformans* (Rónavári et al., 2018; Zhang et al., 2016). Infections caused by viruses are widespread and are getting severe worldwide. Therefore, it is crucial to create anti-viral agents. It was reported that AgNPs exhibited promising antiviral activity due to their unique interaction with viruses (Elechiguerra et al., 2005; Morones et al., 2005). Previous studies have demonstrated that the replication of hepatitis B virus RNA and extracellular virions in vitro can be inhibited by AgNPs (Lu et al., 2008). Besides, a composite consists of AgNPs and chitosan showed antiviral activity against influenza A virus. Greater antiviral activity was observed in smaller size AgNPs (Mori et al., 2013).

2.7.7(c) Wound healing

Healing of wounds and surgical treatment outcomes are tightly connected. Although the recent rapid advancement of nanotechnology has given rise to a new therapeutic approach for the treatment of wounds, further research is still needed on the roles of AgNPs in wound healing. Since AgNPs are well known for their antimicrobial properties, AgNPs used in absorbent wound dressings can prevent wound infection, therefore increase wound healing rate (Kalantari et al., 2020). Additionally, AgNPs can reside in skin biopsies and aid in dermis and epidermis repair, which leads to the restoration of normal skin (Rigo et al., 2013).