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Development of Gold Nanoparticle (AuNP)-based Colorimetric Aptasensor for Penicillin G Detection

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Highlights:

- Optimal conditions for a penicillin G specific aptasensor were found.
- Modification of the penicillin G specific aptamer was done using gold nanoparticles (AuNPs).
- The penicillin G aptasensor could detect penicillin G samples from wild-type and mutant strains of *Penicillium chrysogenum*.

Abstract. Antibiotics are chemical or biological substances that have the ability to kill pathogens selectively. Currently, high-performance liquid chromatography (HPLC) is used routinely in the detection of antibiotics. However, the cost of analysis and running time are bottlenecks for HPLC to be used for routine tests to detect antibiotics. Alternative methods need to be developed to overcome this issue. In this study, the development of a penicillin G specific biosensor by using a DNA aptamer and gold nanoparticles (AuNPs) was done. Optimal aptasensor conditions were achieved with the concentrations of NaCl and aptamer at 0.25 M and 2 μM, respectively. An aptasensor of this type showed LOD for penicillin G at 3 mg/L and was able to detect penicillin G in the range of 3 to 27 mg/L. The established aptasensor showed specific sensitivity toward penicillin G after testing with several antibiotics, i.e., ampicillin, kanamycin, chloramphenicol, and erythromycin. The aptasensor could detect the presence of penicillin G from culture medium of wild-type, ultraviolet irradiation mutant, gamma irradiation mutant, and ultraviolet irradiation and gamma irradiation mutant strains of *P. chrysogenum*, at detection concentrations of 9.75 ± 0.004; 25.25 ± 0.005; 37.5 ± 0.005; and 45 ± 0.004 mg/L, respectively.

Keywords: *aptamer; aptasensor; colorimetry; gold nanoparticles; penicillin G.*

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1 Introduction

Biosensors are instrumentations consisting of two main elements, namely a transduction device and identification elements in the form of enzymes, antibodies or nucleic acids. Aptamers have attracted a great deal of attention from researchers as very promising conventional identification elements [1]. An aptamer is a single-stranded oligonucleotide selected in vitro from a process called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) to recognize target analytes with high specificity and affinity [2]. Aptamers can be single-stranded DNA or RNA sequences and are designed to bind to certain molecules with high specificity and affinity [3]. DNA and RNA aptamers have been developed as sensors for drugs, proteins, antibiotics, organic molecules, and even complex molecules such as viruses or cells [4].

An aptasensor is a type of biosensor in which the biological recognition element used is a DNA or an RNA aptamer. Aptamers are widely utilized in analytical and diagnostic applications. Aptamers have several advantages compared to conventional antibodies, such as better thermostability and more flexibility in designing various types of electrochemical, fluorescence, or colorimetric based biosensors. Among the approaches that have been used in designing aptamers as biosensors is using gold nanoparticles (AuNPs) without modification in the detection process. AuNPs are polymers that can cause discoloration, so they can be used as reagents in optical detection techniques (colorimetry).

Colorimetric detection methods are convenient and effective in various applications because the reading can be done visually without complex instruments [5]. Colorimetric aptasensors based on AuNPs have been developed because of their high detection ability, high sensitivity, and high potential for analysis [6]. Aptasensors can have very high sensitivity, specificity, and reproducibility toward various targets [7]. Using AuNPs in biosensors has several advantages. They have high surface free energy, good biocompatibility, a large surface area so that molecules can be immobilized, and they are catalytically active. Aptamers have been used to detect antibiotics such as chloramphenicol, oxytetracycline, and kanamycin [8].

Antibiotics are chemicals produced by microorganisms that inhibit the growth of microorganisms or kill them. Antibiotics have selective toxicity to one or several destination microorganisms while having weak toxicity to the host (human or animal) [9]. β-lactam class antibiotics are antibiotics commonly used to treat bacterial infections. At present, the consumption of β-lactam antibiotics on the international market is estimated at around 15 billion dollars per year and constitutes 65% of the total antibiotics market [10]. β-lactam antibiotics are widely used as therapeutic agents in the treatment of bacterial infections [11].

AuNP-based colorimetric aptasensors are used to detect small molecules such as antibiotics.

Penicillium chrysogenum is a filamentous fungus (ascomycete) from the Penicillium group that has the ability to synthesize penicillin [12]. Penicillin G belongs to the class of β-lactam antibiotics and has high commercial value because it is widely used to produce other semisynthetic antibiotics such as amoxicillin and ampicillin and because it has the ability to overcome infections caused by bacteria [13]. Penicillin production continues to grow to meet its increasing demand, especially for medical purposes [14]. Increased production of penicillin G can be done by repairing strains with mutations and random screening to get strains with higher production [9].

Currently, the most commonly used methods for detecting antibiotics are high-performance liquid chromatography (HPLC), developed by Mungroo and Neethirajan [15], enzyme-linked immunosorbent assay (ELISA), and thin layer chromatography (TLC) [16]. The latter method is commonly used to detect the presence of penicillin G. However, it requires a long time and is relatively expensive in its application. To support the development of a novel penicillin G detection method that is fast and accurate, the present study focused on the development of a gold nanoparticle-based colorimetric aptasensor for the detection of penicillin G.

2 Material and Methods

2.1 Materials

The materials used were penicillin G binding aptamer sequences (Genetika Science), ultrapure water (Thermo Scientific), NaCl 5M, colloidal gold nanoparticles (AuNPs) with diameter 15 nm (Sigma-Aldrich), and *P. chrysogenum* strains (Institut Pertanian Bogor). The instrument used was an enzyme-linked immunosorbent assay (ELISA) reader. Other equipment used were a laminar airflow cabinet, petri dishes, 96-well plates, a vortex, a micropipette, micropipette tips, an analytical balance, an incubator, and a centrifuge.

2.2 Methods

2.2.1 Optimization of NaCl Concentration

Sterile ultrapure water was added with 62.5 µL colloidal gold nanoparticles (AuNPs). NaCl was added at concentrations of 0.125; 0.25; 0.5; and 1 M, and then incubated for 10 minutes. After incubation, the solution was transferred to a

96-well plate. Sterile ultrapure water was added to reach a final volume of 200 µL and the resulting solution was thoroughly mixed. The absorbance was measured with an ELISA reader at a wavelength of 540 nm [6].

2.2.2 Optimization of Aptamer Concentration

Sterile ultrapure water was added with 62.5 µL colloidal gold nanoparticles (AuNPs) and 0.25 M of NaCl, respectively. Then, aptamer solution (specifically for penicillin G) with varying concentrations (1; 2; 4; and 6 µM) was added and incubated for 10 minutes at room temperature. After incubation, the solution was transferred to a 96-well plate. Sterile ultra-pure water was added to reach a final volume of 200 µL and the resulting solution was thoroughly mixed. The absorbance was measured with an ELISA reader at a wavelength of 540 nm [6].

2.2.3 Detection Limit Test of Aptasensor Penicillin G

Sterile ultrapure water was added with 62.5 µL colloidal gold nanoparticles (AuNPs) and NaCl 0.25 M, respectively. Then, 2 µM of penicillin G binding aptamer solution was added and the resulting solution was incubated for 10 minutes at room temperature. Furthermore, penicillin G was added at various concentrations (1; 3; 9; and 27 mg / L) and incubated for 10 minutes. After incubation, the solution was transferred to a 96-well plate. Sterile ultrapure water was added to reach a final volume of 200 µL and the resulting solution was thoroughly mixed. The absorbance was measured with an ELISA reader at a wavelength of 540 nm [17].

2.2.4 Aptasensor Specificity Test Against Several Antibiotics

Sterile ultrapure water was added with 62.5 µL colloidal gold nanoparticles (AuNPs) and 0.25 M of NaCl, respectively. Then, 2 µM of penicillin G binding aptamer was added and the resulting solution was incubated for 10 minutes at room temperature. Furthermore, penicillin G was added at varied concentrations (1; 3; 9; and 27 mg / L) and incubated for 10 minutes. The solution was then transferred to a 96-well plate. Sterile ultrapure water was added to reach a final volume of 200 µL, after which the solution was thoroughly mixed. The absorbance was measured with an ELISA reader at a wavelength of 540 nm. The aptasensor's selectivity was tested by comparing several antibiotics as controls, namely ampicillin, chloramphenicol, kanamycin and kanamycin [6].

2.2.5 Detection of Penicillin G from *P. Chrysogenum* culture media by Aptasensor Method

Sterile ultrapure water was added with 62.5 µL of gold nanoparticle solution (AuNPs) and 0.25 M of NaCl, respectively. Then, 2 µM of penicillin G binding

aptamer solution was added and the resulting solution was incubated for 10 minutes at room temperature. Penicillin G was added from *P. chrysogenum* wild-type and mutant culture media, and the resulting solution was incubated for 10 minutes and transferred to a 96-well plate. The absorbance was measured with an ELISA reader at a wavelength of 540 nm [6].

3 Result and Discussion

3.1 Optimization of NaCl Concentration

In the development of AuNP based colorimetric aptasensors, the right concentration of NaCl to induce aggregation that results in discoloration after adding targets is determined. According to Katiyar, *et al.* [4], on the surface of the AuNPs, which is negatively charged in the presence of citrate ions, will bind to the positive charge (Na^+) of the NaCl electrolyte. Thus, the nanoparticles have a closer distance to each other, which is visible through color changes caused by a shift in wavelength. This discoloration during aggregation also results in a shift of the plasmon band. Optimization of the NaCl concentration was carried out by using various NaCl concentrations, namely 0.125; 0.25; 0.5 and 1 M.

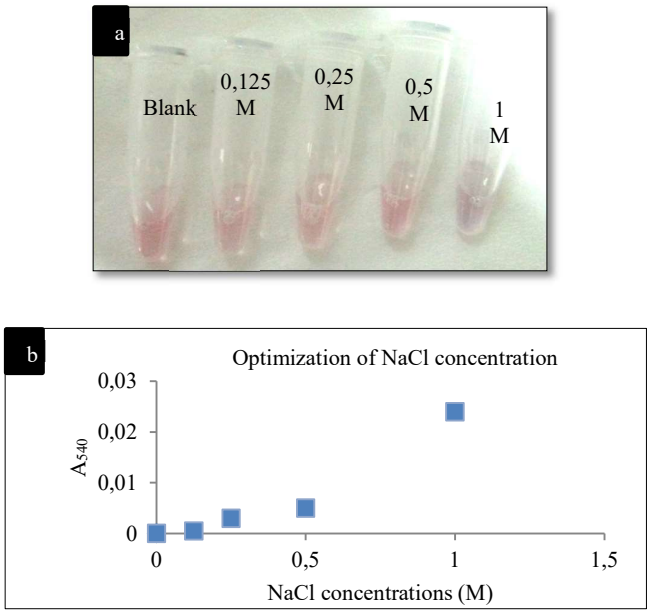

Figure 1 Optimization of NaCl concentration by variation at 0.125; 0.25; 0.5; and 1 M: (a) mixture of solution after incubation, (b) absorption spectra of the AuNP/NaCl solutions with A_{540}.

As shown in Figure 1(a), NaCl with a concentration of 0.25 M is expected to form a red aggregate after the addition of the target (penicillin G) at the end of incubation. Therefore, further experiments were carried out using 0.25 M of NaCl to see the color change. The absorption spectra of the solution measured at a wavelength of 540 nm are shown in Figure 1 (b).

3.2 Optimization of Aptamer Concentration

The penicillin G binding aptamer used was an aptamer that was subjected to the Capture-SELEX process for standard penicillin G developed by Paniel, *et al*. [18]. In the development of the AuNP based colorimetric aptasensor, the minimum aptamer concentration to stabilize the AuNPs was determined. An aptamer concentration that is too high will reduce the sensitivity of the sensor to the target. The aptamer optimization process was carried out by adding AuNPs, NaCl, and penicillin G aptamer. Based on Figure 2(a), the concentration of aptamer was varied, while the amounts of AuNPs and NaCl were kept constant. At an aptamer concentration of 2 µM, the color changed to blue-purple. Therefore, in the next experiment, an aptamer concentration of 2 µM was used.

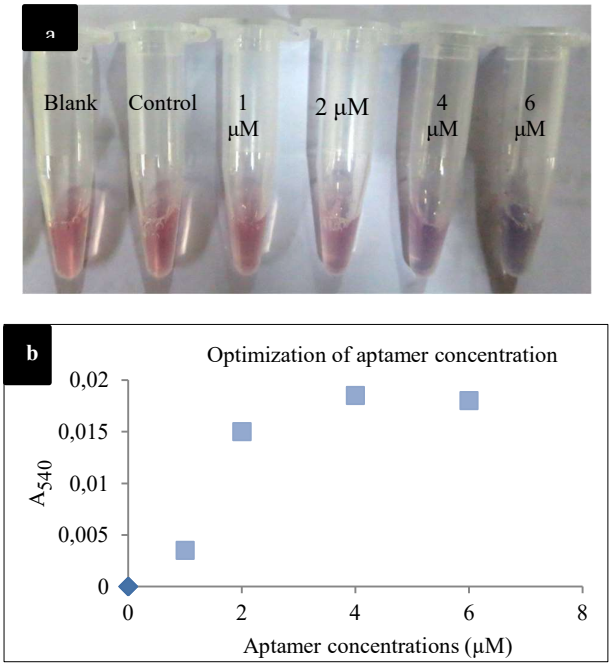

Figure 2 Optimization of aptamer concentration by variation at 1, 2, 4, and 6 µM: (a) mixture of solution after incubation, (b) absorption spectra of AuNP/NaCl/aptamer solutions with A_{540}.

3.3 Detection Limit Test of Penicillin G Aptasensor

Determination of the LOD value and aptasensor linearity was carried out using the optimal concentrations of NaCl and aptamer that had been obtained in the previous procedure. The NaCl and aptamer concentrations used were 0.25 M and 2 µM, respectively. The absorbance was measured at a wavelength of 540 nm with the target (penicillin G) varied at concentrations of 1; 3; 9; and 27 mg/L. With the addition of increasing concentrations of penicillin G, the color became increasingly closer to red. This indicates aggregation between the AuNPs and the NaCl after the addition of the target (penicillin G). Figure 3 shows that the absorbance value of the solution increased with the addition of penicillin G.

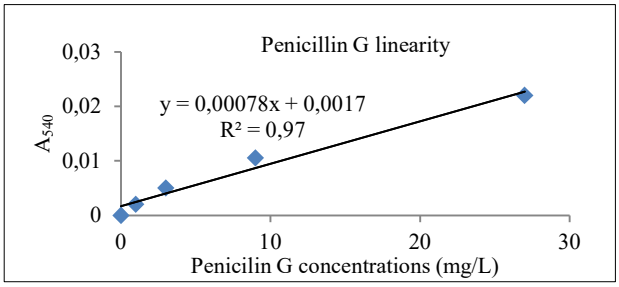

Figure 3 Spectral absorption of the AuNP/NaCl/penicillin G binding aptamer and target (penicillin G) solutions with A_{540}.

The detection limit, determined as the penicillin concentration inducing a response three times higher than the blank variation, was calculated to be 3 mg/L. The optimal condition of the obtained sensor was applied to determine the detection limit of the target (penicillin G). The secondary aptamer structure can bind to various target molecules. According to Acquah, *et al*. in [20], the antibiotic complex can bind to aptamers through several bonds or interactions, namely the installation of non-Watson bases, electrostatic interactions, and intermolecular hydrogen bonds. In this study, there was an electrostatic interaction between the aptamer and the target (penicillin G). Electrostatic interactions occur between the phosphate group in the aptamer and the carbonyl group in the penicillin G structure. The phosphate group is nucleophilic, while the carbonyl group has electrophilic properties.

3.4 Aptasensor Specificity Test Against Several Antibiotics

The colorimetric aptasensor's specificity was tested by comparing the response to 27 mg/L of penicillin G together with several other antibiotics, namely ampicillin, kanamycin, chloramphenicol and erythromycin at the same concentration (Figure 4), after the AuNP/NaCl/aptamer and target (antibiotics)

solution was incubated for 10 minutes to get optimal reaction results from the mixture. Based on the results obtained, the aptamer could bind to penicillin G specifically. The one-way ANOVA test results indicated a statistically significant difference at $p < 0.05$.

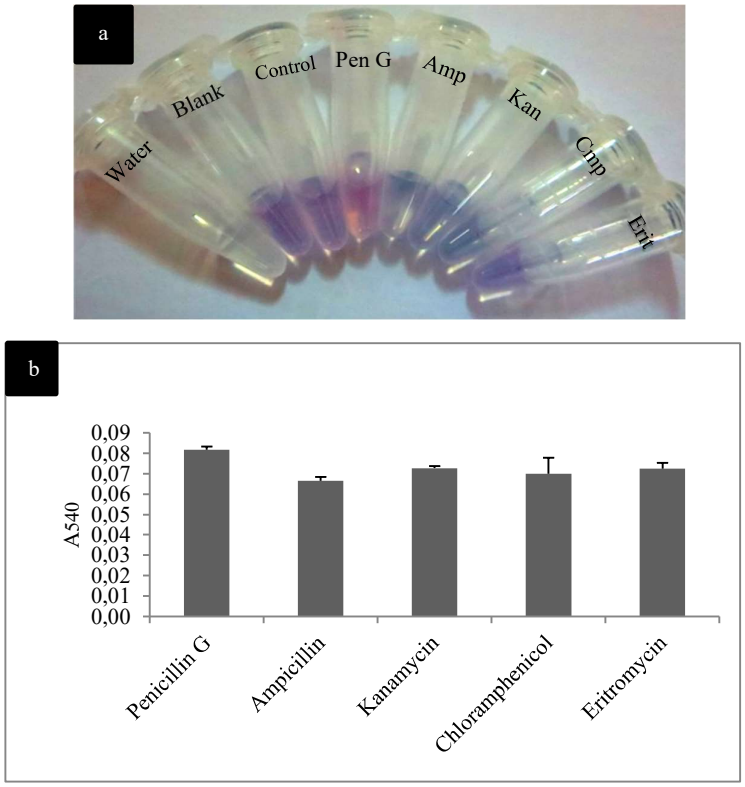

Figure 4 Aptasensor specificity test: (a) solution mixture after incubation, (b) aptasensor absorption of several antibiotic targets.

Bonds were still formed with several other antibiotics, for example with kanamycin. There can be a strong non-specific interaction between kanamycin and the aptamer's DNA structure due to the polycyclic kanamycin structure, which encourages strong interaction with DNA structures (see Paniel, *et al*. [18]). Meanwhile, some other antibiotics could bind to the aptamer because of the similarity of their structure with penicillin G. Figure 4 shows the color changes that occurred in each of the different targets after incubation. According to Huo, *et al.* [21], when the target binds to the aptamer, an aptamer-target complex is formed. Thus, AuNP aggregation occurs, which results in a color change from

blue to red. After the aptamer binds to the target (penicillin G), aggregation is formed, which is marked by a change in color to red. Localized surface plasmon resonance (LSPR) occurs due to an SPR event on the surface of the gold nanoparticles. The free electrons on the surface of the gold nanoparticles resonate at the same frequency as the light waves, resulting in resonant coupling.

3.5 Application of Aptasensor Method

The optimized penicillin G aptasensor was then applied to detect the presence of penicillin G produced by wild-type and mutant strains of *P. chrysogenum* by tests conducted on *P. chrysogenum* culture media. Table 1 shows the results of aptasensor detection against wild-type and mutant strains of penicillin G from *P. chrysogenum.* The penicillin G concentrations detected by the aptasensor increased in the mutants strains compared to the wild-type strains.

Table 1 Results of aptasensor detection of penicillin G from *P. chrysogenum* culture media.

Sample	Penicillin G concentrations (mg / L) ± SD	Increase in penicillin G production
Penicillin G (wild-type)	9.75 ± 0.004	
Penicillin G (UV irradiation mutant)	25.25 ± 0.005	2.6 times
Penicillin G (gamma irradiation mutant)	37.5 ± 0.005	3.8 times
Penicillin G (UV and gamma irradiation mutant)	45 ± 0.004	4.6 times

In the process of detection of penicillin G obtained from the wild-type and mutant strains of *P. chrysogenum*, the color changed from blue to red at the end of incubation. This shows that the optimized aptasensor could detect penicillin G that had been produced. The results obtained indicate that the mutant strains of *P. chrysogenum* were capable of producing more penicillin G when compared to the wild-type strains of *P. chrysogenum*. The aptasensor detection method showed that penicillin G from wild-type, ultraviolet irradiation mutant, gamma irradiation mutant, and ultraviolet irradiation and gamma irradiation mutant strains was present in detected concentrations of 9.75 ± 0.004; 25.25 ± 0.005; 37.5 ± 0.005; and 45 ± 0.004 mg/L, respectively.

4 Conclusions

Optimal aptasensor conditions were achieved with the concentrations of NaCl and aptamer at 0.25 M and 2 μM, respectively. The sensitivity test showed that the limit value for penicillin G aptasensor detection was 3 mg/L. The penicillin

G aptasensor was able to detect penicillin G in the range of 3 to 27 mg/L. It showed specific sensitivity in detecting penicillin G after tests were carried out on several antibiotics. The penicillin G aptasensor developed in this study can be applied for detection of penicillin G obtained from wild-type as well as mutant strains of *P. chrysogenum.*

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