Gold nanoparticle-based detection of *pirA*^{VP} toxin gene causing acute hepatopancreatic necrosis disease (AHPND)

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Abstract

Acute hepatopancreatic necrosis disease (AHPND) caused by *Vibrio* species is one of the most common bacterial diseases carrying the plasmid encoding *pirA*^{VP} and *pirB*^{VP} toxin genes that cause lethargic and anorexic effect on infected shrimps due to induced necrosis in the hepatopancreas. This study demonstrates the use of gold nanoparticles (AuNPs) coupled with specific thiol probe for the detection of *pirA*^{VP} toxin gene causing AHPND. Detection of *pirA*^{VP} toxin gene using thiol probe was validated using colorimetric assay. The validation results showed that the developed Deoxyribonucleic acid (DNA)-based AuNPs detection was specific and highly sensitive to detect the *pirA*^{VP} toxin gene at 20 fg/ul of shrimp genomic DNA. This nanotech-based polymerase chain reaction (PCR) amplification-free protocol can serve as a new means of diagnostic strategy for AHPND detection in aquaculture industry. Furthermore, the method provides accurate and timely detection on field setting which can help to reduce mass mortality and drastic economic losses on aquaculture.

Keywords AHPND · Gold nanoparticles · *PirA*^{VP} toxin gene · Shrimp · Thiol probe

Abbreviations

AHPND	Acute hepatopancreatic necrosis disease
AuNPs	Gold nanoparticles
CTAB	Cetyltrimethylammonium bromide
DTAB	Dodecyltrimethylammonium bromide
PCR	Polymerase Chain Reaction
HAuCl ₄	Gold chloride IV
Na ₃ C ₆ H ₅ O ₇	Sodium citrate

1 Introduction

Shrimp farming in Asia rapidly grows from the traditional polyculture method to export industry for the past decades [5]. Among those cultured shrimp species, Giant tiger prawn (*Penaeus monodon*) of the family Penaeidae was first cultured in earthen ponds of the Philippines in 1950s [27]. As the market demands increases, in 2014, 271 farms which constitutes 3,617.8 hectares of land area were devoted for shrimp farming in the country [3]. This increase yielded a stable annual production of *P. monodon* with approximately 40,000 metric tons from 2011–2016 [21] making it as one of the most cultured decapod in the Philippines [6].

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The main challenge in shrimp production is the occurrence of infectious disease outbreaks causing high mortalities and economic losses. One of the significant non-viral disease in shrimp industry is acute hepatopancreatic necrosis disease (AHPND), formerly known as early mortality syndrome (EMS) causing lethargic and anorexic effect on infected shrimps due to induced necrosis in the hepatopancreas [13]. The AHPND infection also caused notable proximal-to-distal progression of lesion in the hepatopancreas with observed impairment in R-cells (resorptive), B-cells (blister-like) and F-cells (fibrillar) accompanied by acute, massive and progressive degeneration of the HP, followed by a marked reduction of mitotic activity in E-cells (embryonic) [4]. Mortality rates caused by AHPND can reach up to 100% that usually occurs at approximately 35 days after stocking of shrimp postlarvae in ponds [18, 26].

Bacterial pathogen possesses transferrable ~ 69 kbp plasmid carrying the virulent *pirAB*-like toxin genes known as the causative agent of AHPND [2]. Initial identification on the bacterial pathogen causing the disease was *Vibrio parahaemolyticus* which possesses a plasmid with genes that encode homologues of the *Photobacterium* insect-related (Pir) binary toxin, PirA and PirB [11, 12]. However, recent studies showed that this binary toxin is also present in other *Vibrio* spp. [17]. A strain of *Vibrio campbellii* and *Vibrio owensii* were found having the same binary toxins homologous to *pirA*^{VP} / *pirB*^{VP} origin in Vietnam and China respectively [17, 20, 28]. Study conducted by Restrepo et al. [23] also found that even *Vibrio punensis* possess this toxin gene. These findings support the theory that *pirA*^{VP}/*pirB*^{VP} toxin genes are the main virulence factors causing AHPND in shrimp [18].

Health monitoring is an integral part of a successful aquaculture. Hence, a rapid and cheap detection protocol is substantially needed to address this global problem on food security and sustainability. There are efforts to find effective diagnostic methods using molecular approach such as loop-mediated isothermal amplification (LAMP) assay and polymerase chain reaction (PCR) based on targeting the toxin genes *pirA*^{VP} and *pirB*^{VP} for quick detection and prevention of AHPND spread in recent years [14]. However, these techniques still require expensive visualization equipment (e.g., electrophoresis equipment or an ultraviolet (UV) trans-illuminator) and reagents which are not suitable for field testing [2].

In the past years, oligonucleotide functionalized gold nanoparticles (AuNPs) have been demonstrated to be convenient for the detection of nucleic acids [29]. The detection using AuNPs were also found to be efficient without PCR amplification [19] wherein the optical properties of oligonucleotide functionalized AuNPs can be used in colorimetric assays for DNA detection (Doria et al. 2015).

Therefore, the emergence of gold nanoparticles technology can revolutionize disease diagnosis on field condition.

The detection of *pirA*^{VP} toxin gene using AuNPs-based thiol probe could be an alternative to LAMP and PCR, which able to provide rapid, sensitive and specific method for the detection of the pathogen in shrimps. This study developed a convenient and cost-effective diagnostic tool for *pirA*^{VP} toxin gene detection in the field condition that can help to prevent the spread of AHPND in shrimp culture systems, thus giving early preventive solutions and decreasing the chance of mass mortality.

2 Methodology

2.1 Sample preparation and DNA extraction.

Sample preparation, DNA extraction and PCR assay were conducted at BFAR-National Fisheries Laboratory Division at Quezon City, Philippines. About 1 cm of the mid-guts of six juvenile shrimp *Penaeus monodon* (ranging from 5–6 cm) collected from a grow out farm in Zambales, Philippines. The mid-guts samples were used as possible source of *pirA*^{VP} toxin gene. Six shrimp collected from a batch negative for *pirA*^{VP} was cut, macerated and placed into a 1.5 mL tube and centrifuged at 12,000 × g for 3 min to use as tissue samples. The supernatant was discarded and the concentrated pellet was eluted in 600 µL dodecyltrimethylammonium bromide (DTAB) solution.

DNA extraction was carried out using dodecyltrimethylammonium bromide-cetyltrimethylammonium bromide (DTAB-CTAB) DNA extraction kit using manufacturer's protocol. Briefly, the macerated samples were eluted with DTAB solution and incubated at 75°C for 5 min. The mixture was then vortexed and centrifuged for 10 secs at 12,000 × g, added with 0.7 mL chloroform, homogenized using vortex and centrifuged at 12,000 × g for 5 min. Two hundred milliliter of the clear upper aqueous phase of the mixture was pipetted and transferred in another 1.5 mL tube containing 100 µL of CTAB solution and 900 µL ultrapure water. It was then homogenized and incubated at 75°C for 5 min. After incubation, it was cooled down immediately to room temperature and centrifuge at 12,000 × g for 10 min. The supernatant was then discarded and 150 µL of dissolving solution was added, incubated at 75°C for 5 min and centrifuged at 12,000 × g for 5 min. The supernatant which contains the DNA was transferred in a new 1.5 mL tube and precipitated using 300 µL of 95% ethanol by centrifugation for 5 min at 12,000 × g, washed with 200 µL of 75% ethanol and centrifuged at 12,000 × g for 3 min. The tube with DNA pellets was air dried for about 2 h and rehydrated with 200 µL ultrapure water and stored in a – 20°C

refrigerator prior to use. DNA concentration was quantified using spectrophotometer (Nanodrop 2000, Thermo Scientific).

2.2 Polymerase chain reaction (PCR) assay

The verification of positive controls (possible source of *pirA^{VP}* toxin gene) used for specificity testing of the developed thiol probe was carried out using PCR amplification. The primer sequences used in the assay were as follows: 18F 5'-GTGGAAATGGTGAACCTTGC-3' and 45R 5'-CATGTGAGCACCTTCTTAG-3'. A total of 10 µL mixture per reaction contains of 4.6 µL of *sdH₂O*, 3 µL of 10X PCR buffer, 0.5 µL of 25 mM MgCl₂, 0.5 µL of 2 µM dNTPs, 0.2 µL of 10 nM forward and reverse primers and 6–78 ng/µL of the genomic DNA. Amplification was carried out using SimpliAmp Thermal Cycler (Applied Biosystems, USA) with the following conditions: pre-denaturation at 95°C for 5 min and 38 cycles of denaturation at 95°C for 30 secs; annealing 55°C for 30 secs; extension at 72°C for 30 secs and final elongation of 72°C for 5 min.

2.3 Oligonucleotide primers and probe design and synthesis

Specific primers and probe targeting *pirA^{VP}* toxin gene for AHPND detection using AuNP-thiol probe was designed manually using the sequence of *pirA^{VP}* previously deposited in National Center for Biotechnology Information (NCBI). Primers and probe sequences were then checked for loops and self-annealing using OligoAnalyzer (Integrated DNA Technologies). Using the Primer-Blast (NCBI), the specificity of the primers and probe to amplify the correct target gene was verified. Primers were synthesized by Integrated DNA Technologies (USA) while thiol probe was synthesized by Genomics BioSci & Tech (Taiwan) and labeled as *pirA* F/R and AHPND respectively.

2.4 Synthesis of gold nanoparticles

Synthesis of gold nanoparticles was carried out following the protocol of Fernando et al. (2017). Forty-five milliliters of 1 mM gold chloride IV (HAuCl₄) was placed in a clean Erlenmeyer flask and boiled with continuous stirring. Five milliliters of 38.8 mM sodium citrate (Na₃C₆H₅O₇) were used to initiate the reduction process of HAuCl₄. The heating and stirring process were done until the solution turned into wine red color. After confirming the success of the synthesis, the size of the AuNPs was quantified using NanoPartica SZ-100 (Horiba Scientific, United Kingdom).

2.5 Colorimetric detection, specificity and sensitivity testing

The colorimetric detection method of AHPND was based on the study of Ahmadi et al. [1] with modifications. Briefly, a solution was prepared by mixing 1 µL of DNA template, 10 µL of 100 nM probe solution, 10 µL of buffered salt (0.01 M phosphate buffer solution containing 0.2 M NaCl, pH 7.4), 10 µL of 0.25 M sodium chloride (NaCl) solution and 10 µL AuNPs. The solution was then incubated for 20 min at 55 °C. After incubation, 30 µL AuNPs were added to the mixture, followed by checking of the absorbance using UV–Vis spectrophotometer at 525 nm. The changes in absorbance and wavelength were analyzed before and after the colorimetric detection for comparison. Melting temperature for the hybridization process was 57 °C, following the instruction prescribed by Genomics BioSci & Tech (Taiwan). A total volume of 250 nM of AuNPs was used to confirm the result of the colorimetric assay. Hybridization of single-stranded and complementary DNA of *pirA^{VP}* toxin gene in presence of electrolyte will cause the color change of gold nanoparticles (Fig. 1 (Larguinho and Baptista, 2012; Larguinho et al. 2015. Specificity of the DNA-based AuNPs method was tested using the positive and negative control of shrimp samples, which were extracted from the infected and uninfected shrimps respectively. Whereas, sensitivity test was done by means of serial dilution where positive sample and non-vibrio DNA extracts was diluted until 2.0×10^{-5} (20 fg/ul) and were subjected to colorimetric assay. Non-vibrio DNA extracts including bacterial samples of *Staphylococcus haemolyticus* isolate 1, *Staphylococcus haemolyticus* isolate 2, *Plesiomonas shigelloides*, *Staphylococcus arlettae*, *Edwardsiella tarda*, *Bacillus cereus* and *Citrobacter freundii* were also for specificity test.

3 Results

3.1 DNA extraction and polymerase chain reaction assay

All of the extracted samples were subjected to spectrophotometry. DNA concentrations were ranging from 6 to 78 ng/µL. The PCR assay performed to verify positive and negative controls, confirmed that the shrimp samples obtained from Zambales, Philippines grow out farm were positive for *pirA^{VP}* toxin gene. Whereas, the samples obtained from the negative batch confirmed to be negative for *pirA^{VP}* toxin gene. These samples were used in the succeeding experiments for the colorimetric assay using DNA-based AuNPs method.

Fig. 1 Mechanism of DNA-based gold nanoparticle detection of *pirA^{VP}* gene using the DNA-based AuNPs method. The retention of the pink red coloration indicates the absence of the complemen-

tary DNA of the target gene, *pirA^{VP}* (negative) while, the change of color from pink red to purple indicates the presence of the complementary DNA of *pirA^{VP}* toxin gene (positive)

3.2 Synthesis of gold nanoparticles

The synthesized AuNPs were prevalent with a color change from yellow to pink red that occurs after the reduction and capping process of sodium citrate. Formation of AuNPs was quantified with spectrophotometer reading having extinction peak at 525 nm and the particle size of the AuNPs quantified using NanoPartica SZ-100 was 44 nm (Fig. 2a, b). Extinction peak was obtained as a function of the wavelength of the incident radiation that passed through the sample.

3.3 Specificity and sensitivity test through colorimetric detection

Colorimetric detection assay showed positive *pirA^{VP}* detection using DNA-based AuNPs method. All positive control samples (Fig. 3a) changed in color from pink red to purple indicating the positive detection of *pirA^{VP}* toxin gene while the negative controls (Fig. 3b) from uninfected shrimps and non-vibrio DNA extracts including bacterial samples of *Staphylococcus haemolyticus* isolate 1, *Staphylococcus haemolyticus* isolate 2, *Plesiomonas shigelloides*, *Staphylococcus arlettae*, *Edwardshella tarda*, *Bacillus cereus* and *Citrobacter freundii* (Fig. 3c), were detected negative accordingly, retaining its pink red coloration. Specificity and sensitivity test through colorimetric assay were

conducted in 5 replications to assure the reliability of the results. Sensitivity test of DNA-based AuNPs method using colorimetric assay results confirmed that the developed diagnostic tool was able to detect *pirA^{VP}* toxin gene to as low as 20 fg/ul of extracted genomic DNA (Fig. 4). Furthermore, the absorbance of the positive control samples was measured and found to have a significant decrease in absorbance value of 0.048 from 0.210 as compared to the negative controls with 0.137 absorbance value. This means that most of the AuNPs already aggregated because of the presence of *pirA^{VP}* toxin genes in the samples.

4 Discussion

Nanotechnology and its application have been widely explored in the medical field. Advances in disease diagnosis through Nano sensor technologies epitomizes a potential efficiency in healthcare with the existing standard tests [25]. The AuNPs is the most widely used nanoparticles and observed to be different compared to its bulk form having extremely small size and high surface area [15]. This study demonstrates PCR amplification-free method for the detection of AHPND-causing *pirA^{VP}* toxin gene. Presented here is the use of unmodified (no bio-functionalization) AuNPs colorimetric assay which does not rely the verification of results on gel

Fig. 2 **a** synthesized gold nanoparticles (left, resulting pink red solution after the synthesis of gold nanoparticles); gold chloride (HAuCl₄) (right, 1 mM concentration of HAuCl₄). **b** Spectropho-

tometry reading on the wavelength and absorbance of the synthesized gold nanoparticles compared to 1 mM concentration of HAuCl₄ and blank (PCR grade water)

Fig. 3 **a** Positive control samples detected to have *pirA^{VP}* toxin gene using the DNA-based AuNPs method. Color change from pink red to purple indicating the positive detection of *pirA^{VP}* toxin gene (S1-S6), **b** Negative control samples retains its pink red coloration after the colorimetric assay using the DNA-based AuNPs method (N1-N6), **c** Non-vibrio DNA extracts including bacterial samples of *Staphylococcus haemolyticus* isolate 1 (NC1), *Staphylococcus haemolyticus* isolate 2 (NC2), *Plesiomonas shigelloides* (NC3), *Staphylococcus arlettae* (NC4), *Edwardshella tarda* (NC5), *Bacillus cereus* (NC6) and *Citrobacter freundii* (NC7) that showed negative results in DNA-based AuNPS using colorimetric detection assay

Fig. 4 **a** wavelength and absorbance reading of the diluted positive samples from 2.0 ng/ul to 2.0×10^{-5} ng/ul (20 fg/ul) for sensitivity testing using DNA-based AuNPs method, **b** Serially diluted positive samples from 2.0 ng/ul until 2.0×10^{-5} ng/ul for sensitivity test

electrophoresis but instead in the unique property of AuNPs to change its color due to repulsion. One crucial factor is the determination of the amount of phosphate buffer and NaCl relative to amount of AuNPs will indicate a positive result if done correctly. To make an assumption, a mixture containing AuNPs, probe and phosphate buffer must retain its pink red color and surface plasmon resonance even with the presence of NaCl. The red color of AuNPs in buffer solution is highly sensitive to aggregation induced by electrolytes wherein the addition of biomolecule like single stranded DNA (ssDNA) in the solution will create van der Waals interactions. The ssDNA adsorbed onto AuNPs surface adds negative charges on AuNPs surface due to the exposure to phosphate groups making it resistant to aggregation (Ray et al. 2018, [10] (Fig. 1). While, the hybridization of the probe with its complementary DNA will trigger the aggregation of AuNPs which will cause not only the change of color but also the shift of surface resonance.

The samples that retained the pink red color (Fig. 2b) in the assay was due to the conjugation between the probe and AuNPs thereby indicating the absence of the complementary DNA of the target gene, *pirA^{VP}*, making it resistant to aggregation. Whereas, the change in color from pink red to purple in the positive samples (Fig. 2a) indicated the presence of the complementary DNA of *pirA^{VP}* toxin gene, which was hybridized by the probe making the AuNPs aggregate due to the presence of NaCl. The solutions that do not contain the target sequence exhibited no precipitation even when the solution remains overnight at room temperature.

The AuNPs-probe solution exhibits pink red coloration due to its surface plasmon resonance at an absorbance peak of 525 nm. Sodium chloride (NaCl) enhanced precipitation of AuNPs-probe in the presence of a target DNA sequence due to its positively charged property leading to change in color from pink red to purple which shows lower absorbance compared to the negative samples. On the other hand, when a sample does not contain the complementary DNA, the van der Waals interaction will take place. Gold nanoparticles become resistant to aggregation in the presence of electrolytes such as NaCl because of the electrostatic forces between the single-stranded probe and gold nanoparticles. In this study, visual and spectrophotometric comparison of the solutions before and after salt induced AuNPs aggregation clearly established the hybridization success of the AuNPs-probe specific for *pirA^{VP}* toxin gene for AHPND detection.

This DNA-based AuNPs method is completely opposite compared with the method previously developed and optimized by Ganareal et al. [8] as they utilized modified AuNPs thru bio functionalization process. In the case of positive samples for AHPND, coloration stays pink red upon the addition of salt denoting AuNPs-probe stability due to its hybridization with complementary DNA. Hybridization of the thiol probe with its complementary sequence makes the AuNPs resist the aggregation and maintain its pink red coloration. Absorbance peak also maintain its initial value since no color change was observed. Hence, contrary to the positive samples, the negative samples tend to become purple to gray in color that indicates the aggregation between gold nanoparticles. The electrostatic

repulsion between the particles is disrupted during the mixing of solution, therefore causes aggregation of AuNPs (Seetang-Nun et al. 2012). The promising note of this study is the direct utilization of genomic DNA of infected shrimps without PCR amplification, cost effective and rapid detection which is comparable to other molecular assays. Biomonitoring using gold nanoparticle biosensor can be easily done in field settings with the use of simple or alternative heat block for incubation and does not require expensive detection instrumentation. Detection limit for this method can reach up to 20 fg/uL and it exhibits a great specificity of the designed primer sets and probe for colorimetric detection.

5 Conclusion

This study has successfully developed and supports the hybridization procedures of the designed thiol probe for AHPND with its complementary sequence which was found to be highly specific, rapid, cost-effective and highly sensitive to detect 20 fg/uL of AHPND DNA, *pirA*^{VP} toxin gene thus eliminates further detection instrumentation usage. This nanotech-based PCR amplification-free protocol can serve as a new means of diagnostic strategy for AHPND detection in aquaculture industry providing accurate and timely detection on field setting which can reduce mass mortality and drastic economic losses on aquaculture.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest regarding the publication of this article.

References

- Ahmadi S, Kamaladini H, Haddadi F, Sharifmoghdam MR (2018) Thiol-Capped gold nanoparticle biosensors for rapid and sensitive visual colorimetric Detection of *Klebsiella pneumoniae*. J Fluoresc 28(4):987–998. <https://doi.org/10.1007/s10895-018-2262-z>
- Arunrut N, Kampeera J, SIRITHAMMAJAK S, Sanguanrut P, Proespraiwong P, Suebsing R (2016) Sensitive visual detection of ahpnD bacteria using loop-mediated isothermal amplification combined with dna-functionalized gold nanoparticles as probes. PLoS ONE 11(3):e0151769. <https://doi.org/10.1371/journal.pone.0151769>
- Bureau of Fisheries and Aquatic Resources, (2014) Philippines. List of registered aquaculture farms in the Philippines. P 1–20. <http://www.fao.org/fishery/facp/PHL/en>. Accessed 17 Dec 2019
- Devadas S, Banerjee S, Yusoff FM, Bhassu S, Shariff M (2019) Experimental methodologies and diagnostic procedures for acute hepatopancreatic necrosis disease (AHPND). Aquaculture 499:389–400. <https://doi.org/10.1016/j.aquaculture.2018.06.042>
- FAO (Food and Agriculture Organization of the United Nations) (2006) The state of world fisheries and aquaculture. Food and Agricultural Organization of the United Nations, Rome
- FAO 2019. National Aquaculture Sector Overview. Philippines. National Aquaculture Sector Overview Fact Sheets. Text by Paclibare, J.O. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 1 February 2005. [Cited 23 February 2019].
- Fernando SID, De Guia ACM, Gaban PBV, Judan-Cruz KG (2018) Fungicidal activity of gold nanoparticles (AuNPs) formed using Citrate synthesis method against *Candida albicans*. Int J Biol, Pharma and Allied Sci 7(4):507–514. https://ijbpas.com/pdf/2018/April/MS_IJBPA_2018_4392.pdf. Accessed 17 Dec 2019
- Ganareal TACS, Balbin MM, Monserate JJ, Salazar JR, Mingala CN (2018) Gold nanoparticle-based probes for the colorimetric detection of *Mycobacterium avium* subspecies paratuberculosis DNA. Biochem Biophys Res Commun 496:988–997
- Doria G, Baumgartner BG, Franco R, Baptista PV (2010) Optimizing Au-nanoprobes for specific sequence discrimination. Colloids Surf B Biointerfaces 77(2010):122–124. <https://doi.org/10.1016/j.colsurfb.2010.01.007>
- Ghazali Y, Vaseghi A, Ahmadi S, Haddadi F (2018) Simultaneous expression of GUS and Actin genes by using the multiplex RT-PCR and multiplex gold nanoparticle probes. J Fluoresc 28(2):633–638
- Gomez-Gil B, Soto-Rodríguez S, Lozano R, Betancourt-Lozano M (2014) Draft genome sequence of *Vibrio parahaemolyticus* strain M0605, which causes severe mortalities of shrimps in Mexico. Genome Announc 2:e00055–e114
- Gomez-Jimenez S, Noriega-Orozco L, Sotelo-Mundo RR, Cantu-Robles VA, Cobian-Guemes AG, Cota-Verdugo RG, Gamez-Alejo LA, Del Pozo-Yauner L, Guevara-Hernandez E, Garcia-Orozco KD, Lopez-Zavala AA, Ochoa-Leyva A (2014) High-quality draft genomes of two *Vibrio parahaemolyticus* strains aid in understanding acute hepatopancreatic necrosis disease of cultured shrimps in Mexico. Genome Announc 2:e00800–e814
- Hirono I, Tinwongger S, Nochiri Y, Kondo H (2016) Latest research on acute hepatopancreatic necrosis disease (AHPND) of penaeid shrimps. Proceedings of the ASEAN regional technical consultation on EMS/AHPND and other transboundary diseases for improved aquatic animal health in Southeast Asia.
- Hong X, Lu L, Xu D (2016) Progress in research on acute hepatopancreatic necrosis disease (AHPND). Aquac Int 24:577–593
- Khan A, Rashid R, Murtaza G, Zahra A (2014) Gold nanoparticles: synthesis and applications in drug delivery. Trop J Pharm Res 13:1169–1177
- Kimling J, Maier M, Okenve B, Kotaidis V, Ballot H, Plech A (2006) Turkevich method for gold nanoparticle synthesis revisited. J Phys Chem B 110:15700–15707. <https://doi.org/10.1021/jp061667w>
- Kondo H, Van PT, Dang LT, Hirono I (2015) Draft genome sequence of non-*Vibrio parahaemolyticus* acute hepatopancreatic necrosis disease strain KC13.17.5, isolated from diseased shrimp in Vietnam. Genome Announc 3(5):e00978–e1015. <https://doi.org/10.1128/genomeA.00978-15>

18. Lee CT, Chen IT, Yang YT, Ko TP, Huang YT, Huang JY, Huang MF, Lin SJ, Chen CY, Lin SS, Lightner DV, Wang HC, Andrew H, Wang J, Hor LI, Lo CF (2015) The opportunistic marine pathogen *Vibrio parahaemolyticus* becomes virulent by acquiring a plasmid that expresses a deadly toxin. PNAS 112(34):10798–10803
19. Liandris E, Gazouli M, Andreadou M, Čomor M, Abazovic N, Sechi LA, Ikonomopoulos J (2009) Direct detection of unamplified DNA from pathogenic mycobacteria using DNA-derivatized gold nanoparticles. J Microbiol Methods 78:260–264
20. Liu L, Xiao J, Xia X, Pan Y, Yan S, Wang Y (2015) Draft genome sequence of *Vibrio owensii* strain SH-14, which causes shrimp acute hepatopancreatic necrosis disease. Genome Announc 3(6):e01395–e1415. <https://doi.org/10.1128/genomeA.01395-15>
21. Philippine Statistics Authority (2017) Country STAT Philippines Philippines' Shrimp Production in 1996–2016. <https://psa.gov.ph/content/fisheriesstatistics-philippines>. Accessed 17 Dec 2019
22. Ray PC, Fortner A, Griffin J, Kim CK, Singh JP, Yu H (2005) Laser-induced fluorescence quenching of tagged oligonucleotide probes by gold nanoparticles. Chem Phys Lett 414(4):259–264
23. Restrepo L, Bayot B, Arciniegas S, Bajanã L, Betancourt I, Panchana F, Muñoz AR (2018) *pirA*^{VP} genes causing AHPND identified in a new *Vibrio* species (*Vibrio punensis*) within the commensal orientalis clade. Sci Rep 8:13080
24. Seetang-Nun Y, Jaroenram W, Sriurairatana S, Suebsing R, Kiatpathomchai W (2013) Visual detection of white spot syndrome virus using DNA-functionalized gold nanoparticles as probes combined with loop-mediated isothermal amplification. Mol Cell Probes 27:71–79. <https://doi.org/10.1016/j.mcp.2012.11.005>
25. Sin MLY, Mach KE, Wong PK, Liao JC (2014) Advances and challenges in biosensor-based diagnosis of infectious diseases. Expert Rev Mol Diagn 14:225–244
26. Sirikharin R, Taengchaiyaphum S, Sanguanrut P, Chi TD, Mavichak R, Proespraiwong P, Nuangsaeng B, Thitamadee S, Flegel TW, Sritunyalucksana K (2015) characterization and pcr detection of binary, *pir*-like toxins from *vibrio parahaemolyticus* isolates that cause acute hepatopancreatic necrosis disease (AHPND) in Shrimp. PLoS ONE 10:e0126987. <https://doi.org/10.1371/journal.pone.0126987>
27. Villadolid V, Villaluz DK (1951) The cultivation of sugpo (*Penaeus monodon* Fab.) in the Phils. Phil Fish J 1:155–165
28. Xiao J, Liu L, Ke Y, Li X, Liu Y, Pan Y, Yan S, Wang Y (2017) Shrimp AHPND-causing plasmids encoding the *pirAB* toxins as mediated by *pirAB*-Tn903 are prevalent in various *vibrio* species. Sci Rep 7:42177. <https://doi.org/10.1038/srep42177>
29. Zhao W, Lee TMH, Leung SSY, Hsing IM (2007) Tunable stabilization of gold nanoparticles in aqueous solutions by mononucleotides. Am Chem Soc Langmuir 23:7143–7147

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