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Nanostructured Genosensors for Fast Detection of Pathogenic Microorganisms in Water

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Abstract

The integration of nanotechnology, with molecular biology and electrochemistry, have created a great expectation due to the development of new nucleic acid detection strategies based on nanomaterials and which are important tools in genomics, medical diagnosis, pharmacogenetics, pathology, criminology, food security, environmental monitoring, among others; particularly in this stage of the COVID-19 pandemic.

In this work, the DNA detection of four microorganisms was performed: *Escherichia coli*; *Aspergillus niger*; *Salmonella typhi* and *Achlya sp* by electrochemical detection at the DNA level in water, using nanostructured genosensors, which represent a simple device; quick; accurate and economical for the detection of microorganisms.

The specific probes were designed with the primer Blast program, they were synthesized and used with Gold Nanoparticles (AuNPs) as a brand; the probes were functionalized with the AuNPs by the streptavidin-biotin system and hybridization was performed, which was detected electrochemically, and it was observed that the signal decreases when there is less complementarity between the probe and the analytic, with the aim of the developed genosensors to be a tool for the rapid detection of microorganisms for purposes of monitoring, prevention and environmental control among others, like COVID-19; furthermore, if genosensors are produced on a large scale, they can be efficient, inexpensive, portable and simple to use.

Keywords: Genosensors; Microorganisms; DNA probes; Nanoparticles

Introduction

The innovative research that includes disciplines such as molecular biology, electrochemistry, and nanotechnology, has allowed the development of nucleic acid detection strategies that are important in genomics, medical diagnosis, pharmacogenetics, pathology, criminology, food safety, environmental monitoring, among others [1-4].

On another hand, the Nanoparticles (NPs) of noble metals have been studied during the last 25 years, highlighting an important characteristic, its optical resonance, which is in the visible spectrum, as well as its great sensitivity to environmental changes, however for its correct use the protocols specify the importance of their size and shape, which can be sphere, bar, cube, triangle; etc., can also be made functional with a wide range of ligands such as: antibodies, polymers, diagnostic probes, drugs, genetic material, including for the detection of chemical and biological threats [5-13]. Therefore, Gold Nanoparticles (AuNPs) have aroused great interest in various fields of scientific knowledge, especially in studies of biomedical and environmental areas [14-17].

Specifically, for the environmental area, the diagnosis and subsequent recovery of different environments, should be done in addition, considering the implications in ecological and health terms represents the degradation of natural resources. In this sense, microorganisms are good indicators of the trophic status of ecosystems and respond to the disturbances that occurred by modifying their structure in terms of composition and abundance. The detection of changes in aquatic ecosystems, specifically from the communities of fungi, bacteria, and algae, is currently widely used and constitutes a reliable and low-cost mechanism, because it does not require very sophisticated equipment to evaluate the alterations in the systems, since these microorganisms reflect the changes that have occurred in a water resource, unlike the physicochemical parameters that only show the specific situations of the moment of the sample. The indicator microorganisms are those that have a behavior like pathogens, in terms of concentration and reaction to environmental factors, but are easier, faster, and cheaper to identify [18,19].

The conventional techniques of analysis of a specific genetic sequence are based on methodologies of sequencing and hybridization of nucleic acids (DNA and/or RNA). In techniques based on the hybridization of DNA, the sequence of interest or analytic (target) is identified by a probe or oligonucleotide (small DNA sequence of no more than 50 bases), whose sequence is complementary to the analytic; this hybridization reaction occurs with great affinity and specificity. The DNA probes must be marked since the "marks" are responsible for giving the analytical signal to quantify the hybridization event. The use of

DNA associated with different transducers is a new field of research since DNA detection is of interest in genetics, pathology, criminology, environmental monitoring, among others.

Electrochemical genosensors are devices that convert the hybridization event into an electrochemical signal. Advances in the synthesis and characterization of nanostructured materials have produced dramatic changes in the design and capabilities of sensors since devices with electrical, optical, and mechanical properties have been developed; these devices are used to determine the possible interaction of drugs and DNA, as well as in the early and precise diagnosis of infectious agents in different environments. For this, different electrochemical techniques are used: Differential Pulse Voltammetry (DPV), Linear Voltammetry (LV), Square Linear Voltammetry (LSV), Cyclic Voltammetry (VC), among others combined with different electrochemical transducers.

The nanoparticles are used in a wide range of applications, in the biosensors; they are specifically linked to the recognition biomolecule that can be a protein, DNA or RNA. The interaction of streptavidin with biotin is of high affinity and allows that once the complex is formed it is not destabilized, neither by changes in pH nor by multiple washes when immobilized. In this way the recent developments in the design and fabrication of efficient sensor platforms based on nanostructures, such as metal carbon or polymeric nanoparticles, make the highly sensitive sensors which a very low detection limit to the level of few molecules, a genuine possibility [20].

In this work we carried out the molecular identification of the microorganisms *Escherichia coli*, *Aspergillus niger*, *Salmonella typhi* and *Achlya sp.* for the design of specific probes, with gold nanoparticles as a mark to be used in genosensors.

Materials and Methods

All reagents used were analytical grade and all solutions were prepared using double deionized water (Milli-Q, 18 MW cm) from a Millipore purification system.

Obtention of microorganisms

E. coli and *S. typhi*, were donated from the microbiology laboratory of the Professional Biotechnology Unit (UPIBI-IPN), in Mexico city, whereas *Achlya sp* it was isolated from water samples from the Xochimilco canals in Mexico city. 1 ml of the water sample was taken and inoculated in a Petri dish with Sabouraud agar, incubated at 28°C for 48 hours. Subsequently, micro cultures were performed for identification. *Aspergillus niger* it was obtained from compost samples with automotive oil used in the UAM-Azcapotzalco as follows: 10 gr of compost was dissolved in 90 ml of dilution water, 1 mL was taken and inoculated in a Petri dish with Sabouraud agar and incubated at 28°C for 48 hours. Microcultures were performed.

DNA extraction

Once the cultures were pure, the DNA extraction was performed with the MoBioUltraCleanTMsoil DNA Kit, as well as using the extraction protocol described by Dellaporta and Wood, 1983. The obtained DNA was visualized on an Alpha Imager 2000, in 0.8% agarose gels stained with ethidium bromide.

Amplification of DNA by PCR

The DNA obtained from the microorganism was amplified using a thermo cycler Multi Gene Opti Max. The PCR conditions were 95°C for denaturation, 57°C for alignment, 72°C for extension, for 30 cycles using the ITS 4 primers, sequence: 5 'TCCTCCGCTTATTGATATGC 3' and ITS 5, sequence: 5 'GGAAGTAAAAGTCGTAACAAGG 3' [21,22].

Sequencing

The PCR products were sent to the sequencing service of the Molecular Biochemistry Laboratory of the Facultad de Estudios Superiores Iztacala (FES-I) UNAM. With the obtained sequences we proceeded to design the specific probes to each microorganism. Finally, the synthesis of the designed probes, as well as their modification with biotin: probe, probe with 1 error, probe with 3 errors and non-complementary probe, to the commercial house Alpha DNA in Otawa, Canada.

Synthesis of gold nanoparticles (AuNPs

To carry out the synthesis of the AuNPs, the method described by Turkevich, et al. with some modifications. Glass material was used, washed with regia water and Milli-Q water to remove any traces of gold that could interfere during the synthesis process. In a 250 ml Erlenmeyer flask, 500 µL of 1% hydrogen tetrachloroaurate (25 mM) was placed in 50 ml of double deionized water (Milli-Q), it was placed on a heating grate with stirring, until it reached boiling point; subsequently, 1% sodium citrate was added, which acted as a reducing and stabilizing agent [23,24]. They were kept under stirring and heating for no more than 15 minutes. Finally, they allowed to cool to room temperature, whereas maintaining the agitation and kept in a sterile bottle at 4°C for further characterization.

Characterization of AuNPs

The UV-Vis spectroscopic measurements were made using a Perkin Elmer Lambda 25 Spectrophotometer, with a wavelength range of 190-700 nm. The images of Atomic Force Microscopy (AFM) were obtained using a SPM (Scanning Probe Microscope) brand digital instruments. Transmission Electron Microscopy (TEM) images were obtained with a JEOL JEM-100 CX II electron Microscope. The electrochemical characterization of the AuNPs to evaluate the oxidation signal typical of gold reduction was performed by Differential Pulse Voltammetry (DPV) under the following conditions: a sweep of +1.25 V at 0.0 V, potential step 10 mV, amplitude 50 mV, scanning speed 33.5 mvs^{-1} and oxidation time 120 s, in 0.1 M HCl and 0.2 M HCl. The AuNPs were deposited on the surface of the M-GECE electrode, which were left for 5 min. After this time, the electrode was placed in the three-electrode system for analysis by DPV [25].

Functionalization of the probes

For the designed probes to be functional, the streptavidin-biotin system was used. Streptavidin was suspended in sterile Milli Q water to obtain a concentration of 0.1 mg/mL.

The AuNPs synthesized together with the streptavidin solution were placed in 1.5 mL Eppendorf tubes in a ratio of 1:1 (v/v). Subsequently, they were incubated in a thermo shaker (Thermo-Shaker MS-100), for 30 min at 650 rpm and at 25°C.

Immediately after the incubation was finished, they were centrifuged for 20 min at 14000 rpm and at a temperature of 4°C. The supernatant was discarded, and 1 mL of Phosphate Buffer Solution (PBS) was added.

Hybridization and electrochemical detection

The DNA target was added to the solution containing the DNA probe and incubated at 42°C for 15 minutes at 800 rpm in a Thermo-Shaker TS-100, then magnetically separated and the supernatant was decanted. The resulting conjugate was washed twice with 100 µL of TT-buffer and suspended in 20 µL of TTL-buffer, being ready to add the Streptavidin-Activated Gold Nanoparticles (AuNPs/Str) that were used as a label.

The targets used were: Complementary probe, non-complementary probe, probe 1 error, probe 3 errors

The electrochemical detection was carried out by means of Cyclic Voltammetry (VC) analyzing the current-potential response of a polarizable electrode GECE and M-GECE. The electrochemical cell was assembled and filled with 0.2 M hydrochloric acid, the electrodes were connected, a graphite electrode corresponding to the working electrode, one of platinum as against electrode and one of Ag/AgCl as a reference electrode. The sample was placed on the working electrode and a sweep potential of 1.25 mv/s was applied on a Palm Sens computer.

M-GECE electrodes characterization

The electrodes were prepared with epoxy-graphite and incorporating a magneto of neodimium of 3 mm in diameter and 1.5 mm in height. To analyze the surface morphology of the electrodes to know the structure and distribution of the graphite in the paste of the electrode, as well as its roughness, the electrodes were characterized by SEM and FCM. Finally, its electrochemical behavior was evaluated by Cyclic Voltammetry (CV).

Results and Discussion

Isolation of microorganisms

In Figure 1, the micrograph of *Achlya sp* is observed, whereas in Figure 1, *Achlya sp* obtained from the Xochimilco canals in Mexico city is observed. Figure 1 shows the microorganism *Aspergillus niger*, isolated from compost samples.

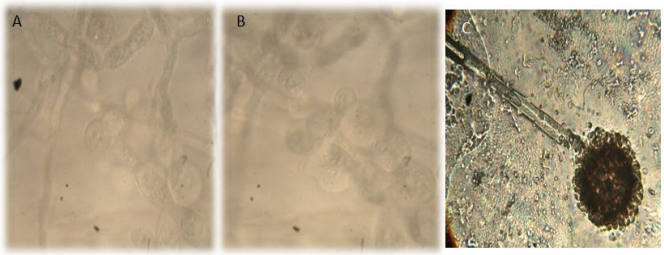

Figure 1: 40 x micrographs, (A). Micrography of *Achlya sp*; (B). Microculture of *Achlya sp.* collected in the Xochimilco canals in Mexico City; (C). *Aspergillus niger* from compost samples.

Design of specific probes

With the sequences obtained and through bioinformatic analysis it was confirmed that the microorganisms of interest (*Achlya sp*) and *Aspergillus niger* were present, the design of the probes was carried out using the primer-blast program. In Table 1 the result is observed.

The designed probes contain high percentage of GC (60%) in order that the complementation in the hybridization was specific and stable.

Table 1: Probes designed by each microorganism.

Microorganism	NCBI/Gene bank	Probe designed
Achlya sp.	JQ974991.1	5' GATCAATACGCCGGTCTCCG 3'
Aspergillus niger.	HQ850370.1	5' CATACGCTCGAGGATCGGAC 3'
E.coli	NZ_AERR00000000.1	5' GCACCGGAAGTACAGACCAA 3'
S. tiphy	FJ460240.1	5' CGGTCGGCTTGAACGAATTG 3'

Synthesis and characterization of Gold Nanoparticles (AuNPs

The synthesized AuNPs were characterized using UV-VIS spectroscopy, Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM), the results of each of them are presented in Figure 2.

For the synthesized AuNPs a maximum wavelength of absorption of 519.5 nm was obtained, this indicates that they are approximately 20 nm, Carralero, 2009 reported a spectrum of 520 nm for these AuNPs.

In the AFM images obtained, AuNPs with a diameter of approximately 20 nm and spherical morphology are observed.

The TEM image of the synthesized AuNPs corroborated the size and shape of the synthesized AuNPs, which are observed in spherical morphology and approximately 20 nm.

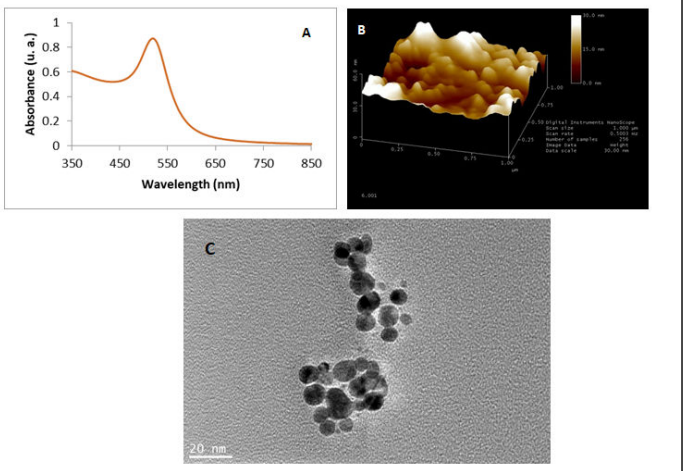

Figure 2: (A). UV-Vis characterization; (B). AFM characterization; (C). TEM characterization.

In the characterization of the AuNPs by Differential Pulse Voltammetry (DPV), a greater response was obtained with the 0.2 M HCl solution (Figure 3).

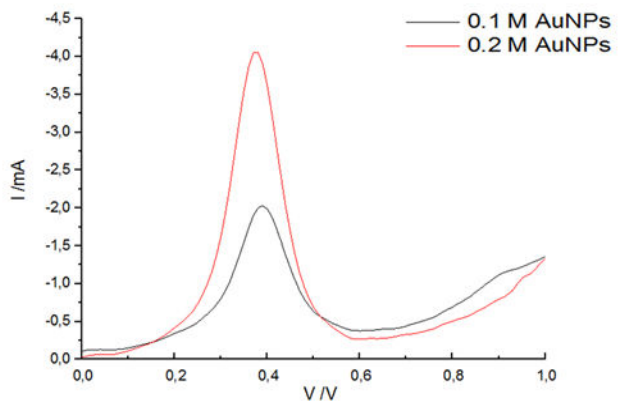

Figure 3: Differential Pulse Voltammetry (DPV) AuNPs at the electrode with 0.1 and 0.2 M HCl.

Characterization of the electrodes by Scanning Electron Microscopy (SEM): The SEM image in the Figure 4 shows the surface of the electrodes, observing that they have an adequate distribution of the epoxy resin and graphite.

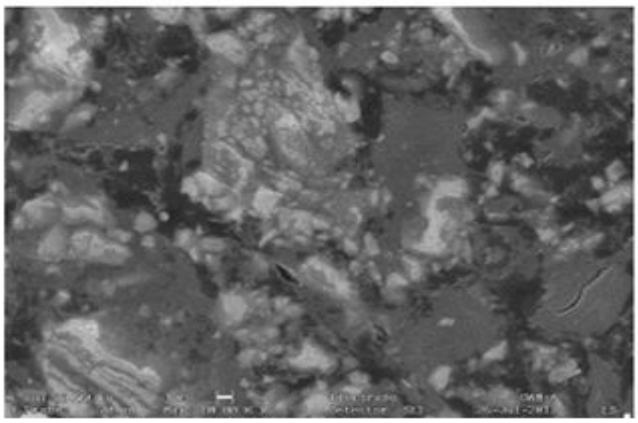

Figure 4: SEM micrographs of the electrode, taken with a resolution of 1 µm and an acceleration potential of 20 KV.

Characterization of the electrodes by Fluorescence Confocal Microscopy (FCM): The electrode surface obtained by FCM is observed uniform in terms of the distribution of the epoxy resin and the graphite (Figure 5).

In the Figure 6 the Cyclic Voltammogram (CV) obtained with the electrode is shown.

This electrode was chosen because it has the fastest oxidation response and gives the best signal.

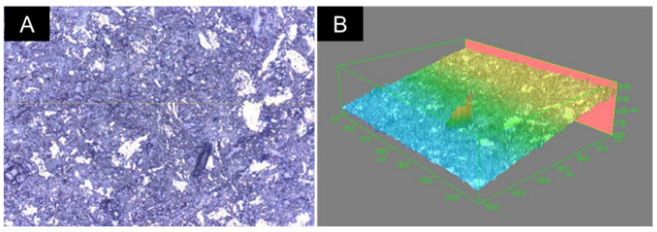

Figure 5: (A). Surface of the electrode by FCM; (B). 3D image where a good roughness of the electrode surface is observed.

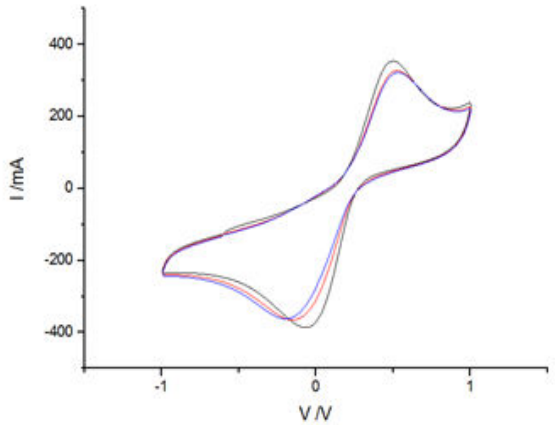

Figure 6: CV voltammograms of the electrode.

Hybridization and electrochemical detection

Hybridization and electrochemical detection were performed, characteristic signals were obtained for each probe: complementary, 1 error, 3 errors and not complementary, it was observed that the signal decreases when there is less complementarity, however it is advisable to perform some optimizations to minimize the signal from the probe not complementary (Figure 7).

It is observed in Figure 8, that the event of 3 errors is greater than 1 error, this may be due to non-controllable or unidentified parameters (Figure 8).

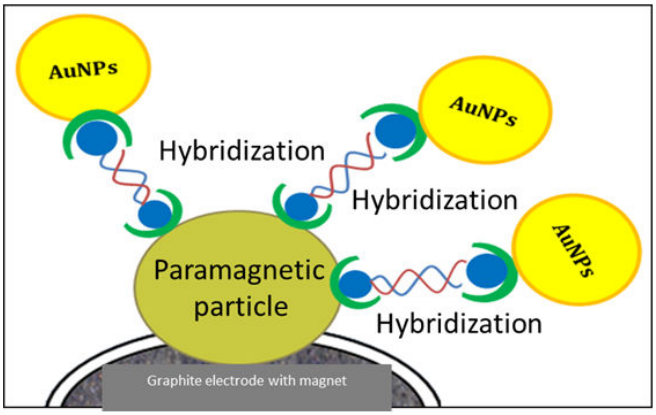

Figure 7: Hybridization event with DNA of the specific probes designed for each microorganism, using the AuNPs brand, for electrochemical detection.

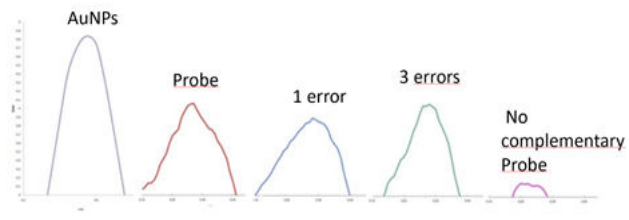

Figure 8: Voltamperograms DPV of the genosensor with the complementary probe, an error, 3 errors and not complementary for *S. typhi*

Electrochemical detection methods make available simple, accurate and inexpensive platform for DNA detection. In addition, the electrochemical genosensors developed in this work, provide direct electronic signal without the use of expensive signal transduction equipment, and facilitates the immobilization of single stranded DNA (ssDNA) probe sequences on an electrode.

Conclusion

Spherical AuNPs of about 20 nm were obtained, which became functional with the specific DNA probes.

The molecular characterization of the microorganisms *Aspergillus sp.* and *Achlya sp.* It allowed identifying at the species level the first, for the second it is necessary to continue sequencing another fragment until obtaining the species. However, the sequence obtained was enough for the design of the specific probes.

Electrochemical detection was achieved for *E. Coli*, *S. Typhi*, *A. niger* and *Achlya sp.* using the specific designed probes and characteristic electrochemical signals were obtained for each probe.

The genosensor developed for each microorganism offers portability, sensitivity, ease of handling, rapid response, small sample volume, low cost and potential application in real samples, environmental samples, and medical samples, among others.

Perspectives

Highly sensitive and stable miniaturized amperometric sensors must have been developed by integrating the nanomaterials and biocatalyst with the transducers. We now are working in the design a biosensor to detect viruses as SARS-CoV-2, among others.

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References

1. Wang J (2003) Nanoparticle-based electrochemical DNA detection. Analytica Chimica Acta 500:247-257
2. Erdem A (2007) Nanomaterial-based elechtrochemical DNA sensing strategies. Talanta 74:318-325
3. Martín-Fernández B, Manzanares-Palenzuela CL, Sánchez-Paniagua López M, de-Los-Santos-Álvarez N, López-Ruiz B (2017) Electrochemical genosensors in food safety assessment. Crit Rev Food Sci 57:2758-2774
4. Chávez-Sandoval BE, Balderas-López JA, García-Franco F, Galindo-Pérez EJ, Martínez-Jiménez A, et al. (2020) The pH role about synthesis, distribution, and potential applications of gold nanoparticles (AuNPs). Int J Biomed Nanosci Nanotech 4:120-138
5. Turkevich P, Stevenson PC, Hilier J (1951) A study of the nucleation and growth process in the synthesis of collidal gold. J Discuss Faraday Soc 11:55-75
6. Sun Y, Xia Y (2002) Shape-controlled synthesis of gold and silver nanoparticle. Science 298: 2176-79.
7. Kimling J, Maier M, Okenve B, Kotaidis V, Ballot H, et al. (2006) Turkevich method for gold nanoparticle. J Phys Chem B 110:15700–15707

8. Castañeda ME, Alegret S, Merkoci A (2007) Electrochemical sensing of dna using gold nanoparticles. Electroanalysis 19:743-753

9. de La Escosura-Muñiz A, Parolo C, Maran F, Mekoçi A (2011) Size-dependent direct electrochemical detection of gold nanoparticles: application in magnetoimmunoassays. Nanoscale 3:3350-3356

10. Chávez-Sandoval BE, Balderas-Lopez JA, Padilla-Bernal G, Moreno-Rivera MA, Franco-Hernandez MO, et al. (2015) Photopyroelectric techniques for thermo-optical characterization of gold nanoparticles. J Phys Conf Ser 582:012027

11. Abrica-González P, Zamora-Justo JA, Chavez-Sandoval BE, Vázquez-Martínez GR, Balderas-López JA (2018) Measurement of the optical properties of gold colloids by photoacoustic spectroscopy. Int J Thermophys 39:1-7

12. Chávez Sandoval BE, Flores-Mendoza N, Chávez-Recio A, Balderas-López JA, García-Franco F (2021) Biosynthesis of gold nanoparticles (AuNPs) and the reducing agents in the process. Mundo nano. Revista Interdisciplinaria En Nanociencias Y Nanotecnología 14: 00036

13. Upadhyayula VK (2012) Functionalized gold nanoparticle supported sensory mechanisms applied in detection of chemical and biological threat agents: A review. Analytica Chimica Acta 715:1-18.

14. Castro-Ortíz LP, Luna Pabello VM, Villalobos Pietrini R (2007) Estado del arte y perspectivas del uso de biosensores ambientales en México. Rev Int Contam Ambie 23:35-45

15. Carralero Sanz V (2009) Preparación de biosensores enzimáticos e inmunosensores basados en electrodos modificados con nanopartículas de oro. Universidad Complutense de Madrid 80-84

16. Wei Y, Han S, Kim J, Soh S, Grzybowski BA (2010) Photoswitchable Catalysis Mediated by Dynamic Aggregation of Nanoparticles. J Am Chem Soc 132:11018–11020

17. Diaz JC, Fontal B, Combita D, Martínez C, Corma A (2011) Synthesis of nano-Au supports and metallic oxides and their catalytic activity and reactions of oxide reduccion de CO. Rev Latin Am Metal Mat (Universidad Simon Bolivar) 33:43-53

18. Chen M, Chen F, Yu Y, Ji J, Kong F (2008) Genetic Diversity of eukariotyc microorganisms in Lake Taihu, a large shallow subtropical lake in China. Microb Ecol 56:572-583

19. Pulido MD, de Navia SL, Torres SM, Prieto AC (2005) Microbiological indicators of contamination of water sources. INOVA (Universidad Colegio Mayor de Cundinamarca, Colombia) 3:69-79

20. Lopez-Marzo AM, Merkoçi A (2016) Paper-based sensors and assays: a success of the engineering design and the convergence of knowledge areas. Lab Chip 16:3150-3176

21. White TJ, Bruns T, Lee SJ, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols, a Guide to Methods and Applications 315-322

22. Faria VV, Rolim LS, Vaz LA, Furtado-Neto MA (2012) Reevaluation of RAPD markers involved in a case of stingray misidentification (Dasyatidae: Dasyatis). Genet Mol Res 11:3835-3845

23. Rasheed PA, Sandhyarani N (2017) Carbon nanostructures as immobilization platform for DNA: A review on current progress in electrochemical DNA sensors. Biosens Bioelectron 97:226-237

24. Estela CB, Angel IM, Francisco G, Jacome GE, Paulina AG, et al. (2016) Biological synthesis and characterization of gold nanoparticles (AuNPs), using plant extracts. J Nanomater Mol Nanotechnol 5

25. Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19-21