# *Artemia franciscana´s* TRANSFORMATION THROUGH BIOLOGICAL SYNTHESIZED GOLD NANOPARTICLES.

Cándido-Mireles Mayra Karina, Aguilar-Méndez Mario Josué, Juárez-Curiel Carlos Alfredo, Rojas-Montoya Aída Bethzabé, Ramírez-Sáenz Diana. Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Ingeniería Campus Guanajuato (UPIIG). Silao de la Victoria, Gto. C.P. 36100, mayrakarina8@hotmail.com

*Keywords: Artemia, bioballistic, nanoparticles.*

**Introduction.** There are previous reports of the transformation of crustacean in which *Artemia franciscana* has been successfully *modified* to express luciferase using bioballistic with chemically synthesized golden nanoparticles (CSGN) (1). However, there are disadvantages in the conventional production of this kind of nanoparticles such as high production costs, production of significant amounts of by-products, among others (2). This research proposes a novel alternative using biologically synthesized gold nanoparticles (BSGN), which production is environmental friendly because the potentially toxic and/or expensive chemicals are reduced (2). Nevertheless it is still necessary to evaluate other characteristics such as high biocompatibility or absence of toxic substances. These characteristics are very important in some applications like bioballistic, which involves the insertion of DNA directly inside the embryo (cyst) nucleus, avoiding its deterioration in the cytoplasm. It is also possible to transform a large number of organisms simultaneously (1).
The objective of this study is to evaluate the use of (BSGN) in *Artemia franciscana* transformation as a possible replacement for CSGN.

**Methods.** CSGN were used as control. BSGN with 3-5 nm of diameter (Nano-Key) were evaluated. Both nanoparticles were washed previously to the DNA bonding according the protocol reported by *Abraham-Juárez* (3). The samples (0.1 g of cyst) were disinfected and treated before the bombardment according to *Gendreau* (1). The transfection experiment was performed with the plasmid pCMV-GFP (addgene). The bombardments were performed at 120 psi, 0.5 cm of distance between the gun barrel and the sample and 0.06 s of opening. ADN and nanoparticles mix (1 µL) were bombarded in each experiment. Subsequently the cysts were cultivated in accordance with *Sorgeloos* (4). The transformation was evaluated for fluorescence microscopy 7 days after the bombardment.

**Results.** Six experiments were performed in separated samples; a negative control, one with CSGN and four with BSGN. All the transformed samples showed the expression of the Green Fluorescence Protein (GFP). Figure 1 shows a comparison of bombarded *Artemias* with CSGN (B), negative control (C). Expression of GFP was positive for B.

Figure 2 confirms the GFP expression in *Artemia* bombarded with BSGN. Both nanoparticles had a visible expression, however are necessary more experiments for quantifiable comparison.

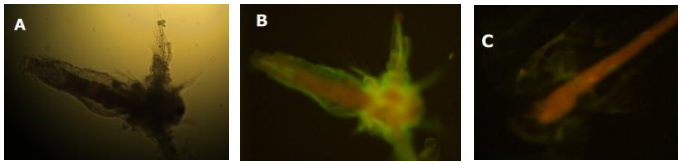


**Fig. 1** 10X Micrography of transformed *Artemia franciscana* with CSGN. (A) Bright field, (B) Fluorescence, (C) negative control, fluorescence.

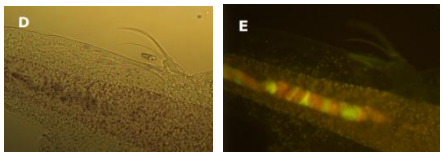


**Fig. 2** 20X Micrography of transformed *Artemia franciscana* with BSGN. (D) Bright field, (E) Fluorescence.

**Conclusions**. Preliminary results confirm the plasmid insertion, through the GFP expression in *Artemia franciscana* bombarded with BSGN, being an effective microprojectile source. Further investigations are necessary to evaluate the transformation efficiency and stability with the use of biologically synthesized nanoparticle.

**Acknowledgment**. We are grateful to Dr. César Aza González, Dr. María del Rosario Abraham Juárez, Dr. Laura Valdés Santiago and to CINVESTAV Irapuato for supplying the manual ballistic device and the fluorescence microscope. This study was funded by CONACyT (project # 191253).

**References.**

1. Gendreau, S., Lardans V., Cadoret J.P. and Miaelhe E. (1995). Transient expression of a luciferase reporter gene after ballistic introduction into Artemia franciscana (Crustacea) embryos. *Aquaculture*. vol (133): 199 – 205.
2. De Wind, W., Vercauteren, T., Verstraete, W. (2011) "Method for producing silver or gold nanoparticles". Europea Patent No. 2,035,567 B1, December 14, 2007.
3. Abraham - Juárez, M. J. (2007). "Estudio de silenciamiento génico en Capsicum: La utilización de un vector geminiviral con secuencias de los genes Comt,pAmt y Kas reduce el contenido de capsaicinoides en los frutos de chile". Tesis de doctorado. Cinvestav - Unidad Irapuato.
4. Sorgeloos, P., Lavens, P., Leger, P., Tackaert, W., Versichele (1986). Manual para el cultivo y uso de *Artemia* en acuacultura. FAO. Brasilia, Brasil.