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Sección: Cartas al editor

Uso de la secuencia del genoma del virus TiLV para desarrollar una vacuna oral recombinante en microalgas. Comentario sobre el artículo “Complete Genome Sequence of a Tilapia Lake Virus Isolate Obtained from Nile Tilapia (Oreochromis niloticus)”

Using the TiLV virus genome sequence to develop a recombinant oral vaccine in microalgae. Comment to the article "Complete Genome Sequence of a Tilapia Lake Virus Isolate Obtained from Nile Tilapia (Oreochromis niloticus)"

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Resumen

La alta mortalidad y diseminación que presenta el virus emergente TiLV (Tilapia Lake Virus) generan la necesidad de desarrollar estrategias para la prevención de la infección en los peces de cultivo. En las enfermedades virales, la vacunación es el método más recomendable para la prevención. En este contexto, y considerando las secuencias del genoma del virus TiLV publicadas en el artículo “Complete Genome Sequence of a Tilapia Lake Virus Isolate Obtained

from Nile Tilapia (*Oreochromis niloticus*)", se propone la producción recombinante de una vacuna oral a través de la biotecnología de microalgas usando vectores virales.
Palabras clave: microalgas; TiLV; vacunas; vector viral

Abstract

High mortality and dissemination rates of the emerging virus TiLV (Tilapia lake Virus) leads the researcher to develop a novel strategies to prevent these kind of infections in the fish. In viral diseases, vaccination is the most recommended method for prevention. In this context and considering the genome sequences of TiLV virus published in the article "Complete Genome Sequence of a Tilapia Lake Virus Isolate Obtained from Nile Tilapia (*Oreochromis niloticus*)", we propose to produce a recombinant oral vaccine through microalgae biotechnology using viral vectors.
Keywords: microalgae; TiLV; vaccine; viral vector

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In 2009, the emergency of a disease capable of causing up to 90 percent mortality in Tilapia fish population was reported in Israel. Later on, in 2014, an enveloped RNA virus denominated Tilapia Lake Virus (TiLV) was associated as the etiological agent (Eyngor *et al*., 2014). Recently, two different groups published the genomic sequences for TiLV (Al-Hussinee *et al*., 2018; Bacharach, *et al*., 2016). Al-Hussinee *et al*., (2018) reported that the TiLV is genetically conformed by 10 segments of RNA in negative sense. Sequence similarity analyses showed that segment 1 codifies for the RNA polymerase enzyme, while the rest of the RNA segments are codifing for hypothetical proteins without similarities to previously reported proteins, and consequently they have unknown functions. Considering that the virus has spread worldwide since its emergence (Al-Hussinee *et al*., 2018), it is compulsory to establish prevention measures in at least two ways: (1) in terms of hygiene and fish-crop management, and (2) vaccination against the TiLV infection. Vaccination approach could consider genomic sequences of TiLV reported by Al-Hussinee *et al*., (2018). Genes for hypothetical TiLV proteins that show similarity with other viral proteins with known immunogenic properties could be heterologously expressed

in microalgae to evaluate their potential as antigens to be used in an oral vaccination strategy. Considering our previous report about the use of viral elements for recombinant protein production in microalgae (Bañuelos-Henández *et al*., 2017), we propose here the development of a geminivirus viral vector for microalgae to allow the cloning of two or three TiLV genes in a single vector. The vector is then use to produce sufficient quantity of recombinant proteins for the vaccination strategy (Fig. 1). TiLV is related to Orthomyxoviruses (Bacharach *et al*., 2016) as is the influenza virus. In influenza virus, the longer proteins (HA, NA, and M1) are main immunogenic antigens (Moon *et al*., 2019; Dukhovlinov *et al*., 2013; Yamashita *et al*., 2010). Based on that information, it could be inferred that the KU751815, KU751816, KU751817, KU751818, KU751819 genes in TiLV may code the homologous immunogenic proteins (HA, NA, and M1).

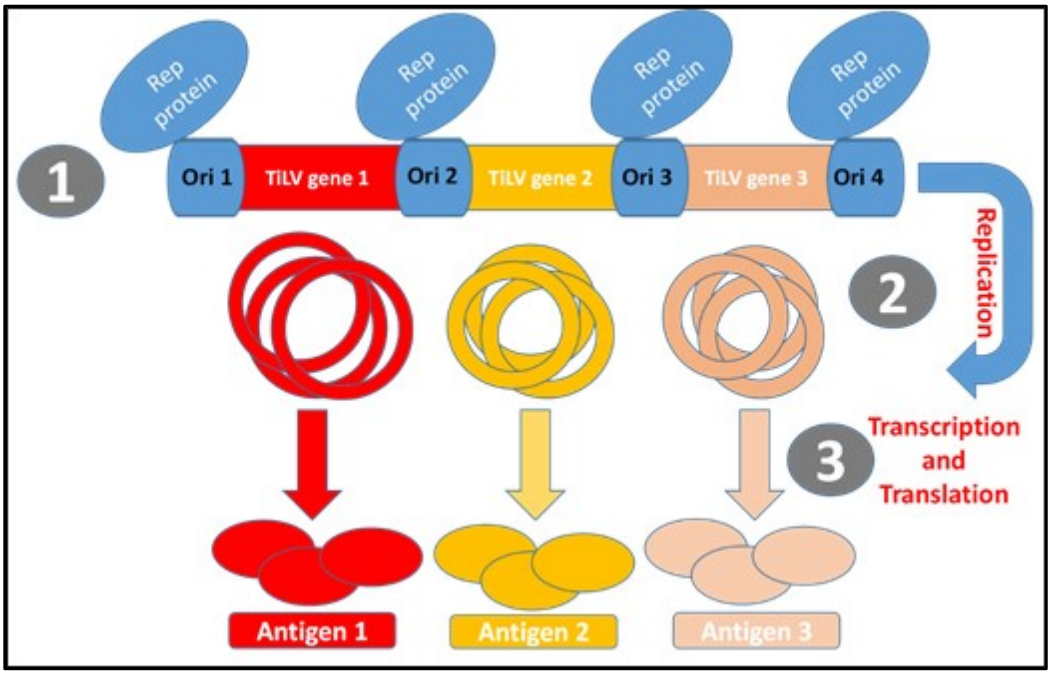

Fig. 1. Schematic representation and functionality of the multigene viral vector for microalgae transformation. 1) Once time viral vector will integrated into the microalgae genome, each one of the TiLV genes (TiLV gene 1-3) will be released by the catalytic action of Rep protein once it bind the replication origins (Ori 1-4), 2) after release, each circular genomic molecules will replicate indepedientelly through rolling circle replication mechanism, 3) high copy number of TiLV genes will produce high quantity of recombinant proteins in microalgae.

Oral inoculation of microalgae producing two or three TiLV antigens is expected to generate protective immunogenicity in the vaccinated fish. On the other hand, antigen bio-encapsulation in the algae cells could improve antigens preservation through the gastrointestinal tract to increase its absorption in small intestine for better bioavailability (Gregory et al., 2013). Recently, the bioabsorption of recombinant protein was evaluated in fish using transgenic microalgae (Chlamydomonas rehinhardtii) as a fish feed (Kwon *et al*., 2019). Microalgae could be considered a good option for antigens production in oral vaccination schemes because tilapia naturally fed microalgae and vegetable detritus; and because the microalgae can be used as an additive in commercial diets (Tesfahun and Temesgen, 2018; Bhujel, 2013; Bowen, 1982). Hypothetically, a 3g fry could ingest 450mg of microalgae containing approximately 250 micrograms of antigen (considering the lowest level of recombinant protein yield using the Algevir system, Bañuelos-Hernández *et al*., 2017).

In conclusion, the availability of public TiLCV genomic sequences and the development of a geminiviral vectors for production of TiLV recombinant proteins in microalgae could contribute to the development of a successful oral vaccine against the TiLV virus.

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