

Draft Genome Sequence of White Spot Syndrome Virus Isolated from Cultured *Litopenaeus vannamei* in Mexico

Libia Zulema Rodriguez-Anaya,^a Jose Reyes Gonzalez-Galaviz,^a Ramon Casillas-Hernandez,^a Fernando Lares-Villa,^a Karel Estrada,^b Jose Cuauhtemoc Ibarra-Gamez,^a Alejandro Sanchez-Flores^b

Departamento de Ciencias Agronómicas y Veterinarias, Instituto Tecnológico de Sonora, Ciudad Obregón, Sonora, Mexico^a; Unidad de Secuenciación Masiva y Bioinformática, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico^b

The first genome sequence of a Mexican white spot syndrome virus is presented here. White spot syndrome is a shrimp pandemic virus that has devastated production in Mexico for more than 10 years. The availability of this genome will greatly aid epidemiological studies worldwide, contributing to the molecular diagnostic and disease prevention in shrimp farming.

Received 4 December 2015 Accepted 29 January 2016 Published 10 March 2016

Citation Rodriguez-Anaya LZ, Gonzalez-Galaviz JR, Casillas-Hernandez R, Lares-Villa F, Estrada K, Ibarra-Gamez JC, Sanchez-Flores A. 2016. Draft genome sequence of white spot syndrome virus isolated from cultured *Litopenaeus vannamei* in Mexico. *Genome Announc* 4(2):e01674-15. doi:10.1128/genomeA.01674-15.

Copyright © 2016 Rodriguez-Anaya et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Jose Cuauhtemoc Ibarra-Gamez, jose.ibarra@itson.edu.mx, or Alejandro Sanchez-Flores, alexsf@ibt.unam.mx.

In Mexico, shrimp farming is the most important aquaculture economic activity. However, its sustainability has been at risk in the last decade due to the low production yields triggered by viral outbreaks (1). Presently, white spot syndrome virus (WSSV) is the most devastating shrimp viral pathogen worldwide, causing mortality of up to 100% of farm production within 2 to 10 days (2). Classified under a new genus, *Whispovirus*, in the family *Nimaviridae* (3), WSSV is extremely virulent and has a wide host tissue tropism (4). Currently, there are four WSSV complete reference genomes deposited in the public databases, with differences in length: WSSV-Taiwan is 307,287 nucleotides (5), WSSV-China is 305,107 nucleotides (6), WSSV-Thailand is 292,967 nucleotides (7), and WSSV-Korea is 295,884 nucleotides (8). However, there is a lack of information related to WSSV functional genomics because of its taxonomical and sequence uniqueness. More than 90% of the predicted open reading frames (ORFs) have no significant similarity to any known proteins (4). Besides, there is evidence that demonstrates the existence of strain variability and differences in pathogenicity among geographical isolates of WSSV (9–13). Therefore, we sequenced, assembled, and analyzed the whole genome of a WSSV Mexican strain (WSSV-MX08), which is the first isolate from Mexican shrimp farms. WSSV-infected shrimp were collected from shrimp ponds in Sonora State. Total DNA was extracted with the GeneJET genomic DNA purification kit, and a library was prepared to be sequenced in the Ion Torrent PGM platform using a 316 Chip, according to the vendor's protocol. We obtained a total of 1,435,498 reads, with a maximum read length of 400 bases, which were assembled in 7,228 contigs. Using the ABACAS (14) and Mauve (15) programs, we aligned the contigs to the four reference genomes available to separate those belonging to WSSV-MX08 from the shrimp genome contigs. Using the alignment information from ABACAS, we reconstructed the whole virus genome by ordering and orienting the contigs, using the WSSV-Thailand reference genome sequence as the template. The G+C content was 41%, which is the same as that of the other WSSV genomes (8), and the average nucleotide identity

(ANI) (16) between WSSV-MX08 and the other genomes is between 99.5% and 99.64%, with the WSSV-Korea genome being the most distant. For coding sequence comparison, we used the Artemis genome browser version 16.0.0 and found a lack of ORFs 122 and 123 in WSSV-MX08, which are also missing in WSSV-Korea (8). The nucleocapsid protein VP35 is also absent, as observed in the WSSV-Thailand (17) and WSSV-Korea (8) strains, suggesting that this protein is not essential for viral replication. One of the biggest difference reported among the isolates is a genetic variation in ORFs 14 and 15 (17), as a 575-bp deletion in this region was observed in WSSV-MX08. In order to understand the evolution of WSSV, further studies are needed to characterize the genomic variations in a protein context and to associate them with other variables, such as the geographical distribution, virulence phenotypes, host-virus interactions, and quasispecies modeling in populations.

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [KU216744](https://www.ncbi.nlm.nih.gov/nuccore/KU216744).

ACKNOWLEDGMENTS

We thank the Unidad de Secuenciación Masiva y Bioinformática, Instituto de Biotecnología (USMB), UNAM, for DNA sequencing advice and bioinformatics analysis. The USMB is part of the Laboratorio Nacional de Apoyo a las Tecnologías en Ciencias Genómicas, which has been created and funded by the CONACYT—Programa de Laboratorios Nacionales.

FUNDING INFORMATION

L.Z. Rodriguez-Anaya was supported by a CONACyT PhD scholarship. Laboratorio Nacional de Apoyo Tecnológico a las Ciencias Genómicas was supported by a CONACyT 260481 grant.

REFERENCES

1. Sanchez-Zazueta E, Martinez-Cordero FJ. 2009. Economic risk assessment of a semi-intensive shrimp farm in Sinaloa, Mexico. *Aquacult Econ Manag* 13:312–327. <http://dx.doi.org/10.1080/13657300903351685>.
2. Sablok G, Sánchez-Paz A, Wu X, Ranjan J, Kuo J, Bulla I. 2012. Genome

- dynamics in three different geographical isolates of white spot syndrome virus (WSSV). *Arch Virol* 157:2357–2362. <http://dx.doi.org/10.1007/s00705-012-1395-7>.
3. Vlak JM, Bonami JR, Flegel TW, Kou GH, Lightner DV, Loh CF, Loh PC, Walker PW. 2005. Nimaviridae, p 187–192. In Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (ed), *Virus taxonomy*, 8th report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London, United Kingdom.
 4. Liu KF, Liu W, Kou G, Lo C. 2009. Shrimp white spot syndrome—from pathology to pathogenomics. *Fish Pathol* 44:55–58. <http://dx.doi.org/10.3147/jsfp.44.55>.
 5. Tsai MF, Yu HT, Tzeng HF, Leu JH, Chou CM, Huang CJ, Wang CH, Lin JY, Kou CH, Lo CF. 2000. Identification and characterization of a shrimp white spot syndrome virus (WSSV) gene that encodes a novel chimeric polypeptide of cellular-type thymidine kinase and thymidylate kinase. *Virology* 277:100–110. <http://dx.doi.org/10.1006/viro.2000.0597>.
 6. Yang F, He J, Lin X, Li Q, Pan D, Zhang X, Xu X. 2001. Complete genome sequence of the shrimp white spot bacilliform virus. *J Virol* 75:11811–11820. <http://dx.doi.org/10.1128/JVI.75.23.11811-11820.2001>.
 7. Van Hulten MC, Witteveldt J, Peters S, Kloosterboer N, Tarchini R, Fiers M, Sandbrink H, Lankhorst RK, Vlak JM. 2001. The white spot syndrome virus DNA genome sequence. *Virology* 286:7–22. <http://dx.doi.org/10.1006/viro.2001.1002>.
 8. Chai CY, Yoon J, Lee YS, Kim YB, Choi TJ. 2013. Analysis of the complete nucleotide sequence of a white spot syndrome virus isolated from Pacific white shrimp. *J Microbiol* 51:695–699. <http://dx.doi.org/10.1007/s12275-013-3171-0>.
 9. Wongteerasupaya C, Pungchai P, Withyachumnarnkul B, Boonsaeng V, Panyim S, Flegel TW, Walker PJ. 2003. High variation in repetitive DNA fragment length for white spot syndrome virus (WSSV) isolates in Thailand. *Dis Aquat Org* 54:253–257. <http://dx.doi.org/10.3354/dao054253>.
 10. Dieu BT, Marks H, Siebenga JJ, Goldbach RW, Zuidema D, Duong TP, Vlak JM. 2004. Molecular epidemiology of white spot syndrome virus within Vietnam. *J Gen Virol* 85:3607–3618. <http://dx.doi.org/10.1099/vir.0.80344-0>.
 11. Waikhom G, John KR, George MR, Jeyaseelan MJP. 2006. Differential host passaging alters pathogenicity and induces genomic variation in white spot syndrome virus. *Aquaculture* 261:54–63. <http://dx.doi.org/10.1016/j.aquaculture.2006.07.031>.
 12. González-Galaviz JR, Rodríguez-Anaya LZ, Molina-Garza ZJ, Ibarra-Gómez JC, Galaviz-Silva L. 2013. Genotyping of white spot syndrome virus on wild and farm crustaceans from Sonora, Mexico. *Arch J Biol Sci* 65:945–947.
 13. de Jesús Durán-Avelar M, Pérez-Enríquez R, Zambrano-Zaragoza JF, Montoya-Rodríguez L, Vázquez-Juárez R, Vibanco-Pérez N. 2015. Genotyping WSSV isolates from northwestern Mexican shrimp farms affected by white spot disease outbreaks in 2010–2012. *Dis Aquat Org* 114:11–20. <http://dx.doi.org/10.3354/dao02844>.
 14. Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. 2009. ABACAS: algorithm-based automatic contiguation of assembled sequences. *Bioinformatics* 25:1968–1969.
 15. Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res* 14:1394–1403. <http://dx.doi.org/10.1101/gr.2289704>.
 16. Rodríguez RLM, Konstantinidis KT. 2014. Bypassing cultivation to identify bacterial species. *Microbe* 9:111–118.
 17. Marks H, Goldbach RW, Vlak JM, van Hulten MC. 2004. Genetic variation among isolates of white spot syndrome virus. *Arch Virol* 149:673–697. <http://dx.doi.org/10.1007/s00705-003-0248-9>.