

Complete Genome Sequences of *Helicoverpa armigera* Single Nucleopolyhedrovirus Strains AC53 and H25EA1 from Australia

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We report here the genome sequences of two alphabaculoviruses of *Helicoverpa* spp. from Australia: AC53, used in the biopesticides ViVUS and ViVUS Max, and H25EA1, used in *in vitro* production studies.

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Helicoverpa spp. (Lepidoptera, Noctuidae) are polyphagous pests of international significance (1). Widespread resistance to chemical insecticides has prompted the registration of biopesticides based on baculoviruses (*Baculoviridae*) (2).

Two species of group II nucleopolyhedroviruses (genus *Alphabaculovirus*) from *Helicoverpa* species have been designated *Helicoverpa armigera* single nucleopolyhedrovirus (HaSNPV) and *Helicoverpa zea* single nucleopolyhedrovirus (HzSNPV) (3–11). Strain AC53 (also known as A44WT [11–13]) is used in the biopesticides ViVUS and ViVUS Max (AgBiTech Pty. Ltd.) (2). It was originally isolated from an unspecified *Helicoverpa* species from a cadaver from Brookstead, Southeast Queensland, Australia, in 1974 (2, 11–13) and isolate P9/H25WT from an unspecified *Helicoverpa* species from a cadaver from Central Queensland in 1973 (14–19). Both isolates were passaged initially through *Helicoverpa punctigera* Wallengren and then repeatedly through *H. armigera* (Hübner) by the Queensland Department of Primary Industries (DAFF Qld); strain H25EA1, used *in vitro* baculovirus production, was selected *in vitro* by CSIRO from P9/H25WT (14–19).

AC53 (AgBiTech Pty. Ltd.) and H25EA1 (from S. Reid, University of Queensland) were passaged once through *H. armigera* larvae. Viral DNA was extracted from occlusion bodies, as previously described (7, 20), and sequenced using the Ion Torrent PGM (316 Chip, 200-bp chemistry). Read quality was determined using FastQC 0.11.2 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and the qProfiler tool from the AdamaJava project (Queensland Centre for Medical Genomics) and trimmed using CLC Genomics Workbench 7.04 (CLC 7.04), with a final Phred score of 28.

AC53 contigs were assembled *de novo* using CLC 7.04 and compared with BLAST against all available *Helicoverpa* species SNPV genomes (GenBank accession numbers JN584482, NC011354, NC003349, NC003094, and NC002645). The HzSNPV (accession no. NC003349) genome was selected as a mapping reference and a consensus sequence for AC53 produced using Burrows-Wheeler aligner (BWA)-mem 0.7.5a, SAMtools 0.19, and the genome analysis toolkit GATK 3.1-1. *De novo* contigs were assembled to fill

gaps (21–25). Assembly of the H25EA1 genome was conducted according to the same process for mapping to the AC53 sequence.

The AC53 and H25EA1 genomes were, 130,442 bp and 130,440 bp, with G+C contents of 39.2% and 39.1%, respectively. The homology between the strains was 99.60%. The homology to HzSNPV (accession no. NC003349) was 99.56% but ranged between 98.43% (accession no. NC003094) and 98.99% (accession no. NC011354) in comparison to HaSNPV genomes.

Both strains contain 138 open reading frames (ORFs), 5 homologous repeat (Hr) regions, and all 62 of the conserved genes were found in all lepidopteran baculoviruses (26). Of the 138 ORFs, 52 had 100% sequence homology between the two strains. The greatest differences between AC53 and H25EA1 were found in the baculovirus repeated open reading frames BRO-A (89.78% homology), and BRO-B (96.41% homology) and the 5 Hr regions (94.61% to 99.27%). However, they contained 100% homology in the BRO region located at ORF107. This is consistent with many baculoviruses (27–29).

Both isolates contained the HaSNPV ORF42 (typically located at ORF43 in HzSNPVs) (30, 31), but unlike published HaSNPV genomes, both contained the HzSNPV ORF79 (7, 28), located at ORF78.

We conclude that AC53 and H25EA1 are type II *Heliothine* SNPVs intermediate between HzSNPV and HaSNPV and support the argument that all *Heliothine* SNPVs are variants of a single species of HaSNPV (3–5, 7).

Nucleotide sequence accession numbers. The complete sequences of HaSNPV AC53 and HaSNPV H25EA1 were deposited to GenBank under the accession numbers [KJ909666](https://www.ncbi.nlm.nih.gov/nuccore/KJ909666) and [KJ922128](https://www.ncbi.nlm.nih.gov/nuccore/KJ922128), respectively.

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REFERENCES

- Tay WT, Soria MF, Walsh T, Thomazoni D, Silvie P, Behere GT, Anderson C, Downes S. 2013. A brave new world for an old world pest: *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. *PLoS One* 8:e80134. <http://dx.doi.org/10.1371/journal.pone.0080134>.
- Buerger P, Hauxwell C, Murray D. 2007. Nucleopolyhedrovirus introduction in Australia. *Virol Sin* 22:173–179. <http://dx.doi.org/10.1007/s12250-007-0019-y>.
- Jehle JA, Lange M, Wang H, Hu Z, Wang Y, Hauschild R. 2006. Molecular identification and phylogenetic analysis of baculoviruses from *Lepidoptera*. *Virol* 346:180–193. <http://dx.doi.org/10.1016/j.virol.2005.10.032>.
- Jehle JA, Blissard GW, Bonning BC, Cory JS, Herniou EA, Rohrmann GF, Theilmann DA, Thiem SM, Vlak JM. 2006. On the classification and nomenclature of baculoviruses: a proposal for revision. *Arch Virol* 151:1257–1266. <http://dx.doi.org/10.1007/s00705-006-0763-6>.
- Herniou EA, Jehle JA. 2007. Baculovirus phylogeny and evolution. *Curr Drug Targets* 8:1043–1050. <http://dx.doi.org/10.2174/138945007782151306>.
- Chen X, Zhang WJ, Wong J, Chun G, Lu A, McCutchen B, Presnail J, Herrmann R, Dolan M, Tingey S. 2002. Comparative analysis of the complete genome sequences of *Helicoverpa zea* and *Helicoverpa armigera* single-nucleocapsid nucleopolyhedroviruses. *J Gen Virol* 83:673–684.
- Rowley DL, Popham HJ, Harrison RL. 2011. Genetic variation and virulence of nucleopolyhedroviruses isolated worldwide from the *Heliothis* pests *Helicoverpa armigera*, *Helicoverpa zea*, and *Heliothis virescens*. *J Invertebr Pathol* 107:112–126. <http://dx.doi.org/10.1016/j.jip.2011.03.007>.
- Wardhaugh KG, Room PM, Greenup LR. 1980. The incidence of *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae) on cotton and other host-plants in the Namoi valley of New South Wales. *Bull Entomol Res* 70:113–131. <http://dx.doi.org/10.1017/S0007485300009822>.
- Daly JC, Gregg P. 1985. Genetic variation in *Heliothis* in Australia: species identification and gene flow in the two pest species *H. armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae). *Bull Entomol Res* 75:169–184. <http://dx.doi.org/10.1017/S0007485300014243>.
- Zhang G. 1989. Commercial viral insecticide *Heliothis armigera* viral insecticide in China. *The IPM Practitioner* 11:13.
- Richards AR, Christian PD. 1999. A rapid bioassay screen for quantifying nucleopolyhedroviruses (*Baculoviridae*) in the environment. *J Virol Methods* 82:63–75. [http://dx.doi.org/10.1016/S0166-0934\(99\)00080-4](http://dx.doi.org/10.1016/S0166-0934(99)00080-4).
- Richards A, Cory J, Speight M, Williams T. 1999. Foraging in a pathogen reservoir can lead to local host population extinction: a case study of a *Lepidoptera*-virus interaction. *Oecologia* 118:29–38. <http://dx.doi.org/10.1007/s004420050700>.
- Christian PD, Gibb N, Kasprzak AB, Richards A. 2001. A rapid method for the identification and differentiation of *Helicoverpa* nucleopolyhedroviruses (NPV *Baculoviridae*) isolated from the environment. *J Virol Methods* 96:51–65. [http://dx.doi.org/10.1016/S0166-0934\(01\)00318-4](http://dx.doi.org/10.1016/S0166-0934(01)00318-4).
- Lua LH, Reid S. 2000. Virus morphogenesis of *Helicoverpa armigera* nucleopolyhedrovirus in *Helicoverpa zea* serum-free suspension culture. *J Gen Virol* 81:2531–2543.
- Lua LH, Pedrini MR, Reid S, Robertson A, Tribe DE. 2002. Phenotypic and genotypic analysis of *Helicoverpa armigera* nucleopolyhedrovirus serially passaged in cell culture. *J Gen Virol* 83:945–955.
- Pedrini MR, Christian P, Nielsen LK, Reid S, Chan LC. 2006. Importance of virus–medium interactions on the biological activity of wild-type *Heliothis* nucleopolyhedroviruses propagated via suspension insect cell cultures. *J Virol Methods* 136:267–272.
- Nguyen Q, Qi YM, Wu Y, Chan LC, Nielsen LK, Reid S. 2011. *In vitro* production of *Helicoverpa baculovirus* biopesticides—automated selection of insect cell clones for manufacturing and systems biology studies. *J Virol Methods* 175:197–205. <http://dx.doi.org/10.1016/j.jviromet.2011.05.011>.
- Nguyen Q, Nielsen LK, Reid S. 2013. Genome scale transcriptomics of baculovirus-insect interactions. *Viruses* 5:2721–2747. <http://dx.doi.org/10.3390/v5112721>.
- Matindoost L, Nielsen LK, Reid S. 2015. Intracellular trafficking of baculovirus particles: a quantitative study of the HearNPV/HzAM1 cell and AcMNPV/Sf9 cell systems. *Viruses* 7:2288–2307. <http://dx.doi.org/10.3390/v7052288>.
- Baillie VL, Bouwer G. 2011. Development of highly sensitive assays for detection of genetic variation in key *Helicoverpa armigera* nucleopolyhedrovirus genes. *J Virol Methods* 178:179–185. <http://dx.doi.org/10.1016/j.jviromet.2011.09.009>.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25:2078–2079. <http://dx.doi.org/10.1093/bioinformatics/btp352>.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 20:1297–1303. <http://dx.doi.org/10.1101/gr.107524.110>.
- DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, McKenna A, Fennell TJ, Kernysky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 43:491–498. <http://dx.doi.org/10.1038/ng.806>.
- Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, del Angel G, Levy-Moonshine A, Jordan T, Shakir K, Roazen D, Thibault J, Banks E, Garimella KV, Altschuler D, Gabriel S, DePristo MA. From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Curr Protoc Bioinformatics* 11:11.10.1–11.10.33. <http://dx.doi.org/10.1002/0471250953.bi1110s43>.
- Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25:1754–1760. <http://dx.doi.org/10.1093/bioinformatics/btp324>.
- Herniou EA, Olszewski JA, Cory JS, O'Reilly DR. 2003. The genome sequence and evolution of baculoviruses. *Annu Rev Entomol* 48:211–234. <http://dx.doi.org/10.1146/annurev.ento.48.091801.112756>.
- Bidshi DK, Renault S, Stasiak K, Federici BA, Bigot Y. 2003. Phylogenetic analysis and possible function of bro-like genes, a multigenic family widespread among large double-stranded DNA viruses of invertebrates and bacteria. *J Gen Virol* 84:2531–2544. <http://dx.doi.org/10.1099/vir.0.19256-0>.
- Le TH, Wu T, Robertson A, Bulach D, Cowan P, Goodge K, Tribe D. 1997. Genetically variable triplet repeats in a RING-finger ORF of *Helicoverpa* species baculoviruses. *Virus Res* 49:67–77. [http://dx.doi.org/10.1016/S0168-1702\(97\)01454-8](http://dx.doi.org/10.1016/S0168-1702(97)01454-8).
- Erlandson MA. 2009. Genetic variation in field populations of baculoviruses: mechanisms for generating variation and its potential role in baculovirus epizootiology. *Virol Sin* 24:458–469. <http://dx.doi.org/10.1007/s12250-009-3052-1>.
- Chen X, Ijkel WF, Tarchini R, Sun X, Sandbrink H, Wang H, Peters S, Zuidema D, Lankhorst RK, Vlak JM. 2001. The sequence of the *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus genome. *J Gen Virol* 82:241–257.
- Chen X, Zhang W-J, Wong J, Chun G, Lu A, McCutchen B, Presnail J, Herrmann R, Dolan M, Tingey S. 2002. Comparative analysis of the complete genome sequences of *Helicoverpa zea* and *Helicoverpa armigera* single-nucleocapsid nucleopolyhedroviruses. *J Gen Virol* 83:673–684.