



Isolation of Two Virus-Like Circular DNAs from Commercially Available Milk Samples

Konstantina Falida, Sebastian Eilebrecht, Karin Gunst, Harald zur Hausen, Ethel-Michele de Villiers

Division of Episomal-Persistent DNA in Cancer and Chronic Diseases, Deutsches Krebsforschungszentrum, Heidelberg, Germany

ABSTRACT Epidemiological data indicate a potential relationship between milk and dairy product consumption and the incidence of breast cancer, as well as neurodegenerative diseases. We report the isolation of two novel circular DNA molecules isolated from commercially available milk.

A number of studies link the consumption of milk and dairy products with the incidence of breast cancer, as well as of neurodegenerative diseases, such as multiple sclerosis (1, 2). Eighteen novel circular DNA molecules were recently isolated from cow milk, bovine sera, and blood and tissue samples from multiple sclerosis patients (3–6). We extended these studies to the analyses of additional dairy milk samples, as well as other dairy products, i.e., six commercially available dairy milk samples and two samples each from yogurt, crème fraîche, curd cheese, and butter.

DNA was extracted with phenol-chloroform and subjected to rolling-circle amplification (RCA) using random primers. Resulting products were amplified by PCR using specific abutting primers, as previously described (3), targeting a highly conserved region in the replication gene. PCR products were cloned into pCR2.1 vector (Invitrogen) prior to sequencing by primer walking. We isolated two novel circular DNA sequences from one dairy milk sample, cow milk isolate (CMI) 5.170 (1,706 bp) and CMI5.240 (2,406 bp), sharing 89% and 68% nucleotide identity, respectively, to multiple sclerosis brain isolate (MSBI) 1.176 (1,766 bp) (3). The open reading frame (ORF) encoding a putative replication protein (324 amino acids [aa]) is highly conserved between these isolates, with only a 1-amino-acid difference between the putative Rep protein of CMI5.170, and a 3-amino-acid difference in CMI5.240 compared to that of MSBI1.176. A poly(A) for each of these ORFs is located at nucleotides (nt) 1637 to 1642 (CMI5.170) and nt 1927 to 1932 (CMI5.240). Repeat regions (22 nt × 4) are present in analogy to previous isolates (3). CMI5.240 contains an additional larger ORF (125 aa) in an antisense direction, which shares 96% amino acid identity to a similar antisense-directed ORF in CMI2.214 (3).

Furthermore, we demonstrated the presence of three previously isolated agents in additional dairy products. CMI3.168 was isolated from milk, yogurt, and curd cheese, CMI4.158 from milk, and healthy cattle blood isolate (HCBI) 6.252 from milk and yogurt (3).

Accession number(s). The complete sequences of CMI5.170 and CMI5.240 have been deposited in the EMBL database under the accession numbers [LT715554](https://www.ebi.ac.uk/EMBL/nuclseq/LT715554) and [LT715555](https://www.ebi.ac.uk/EMBL/nuclseq/LT715555), respectively.

ACKNOWLEDGMENT

This study was supported by an unrestricted grant from ORYX, Munich, Germany.

Received 6 March 2017 **Accepted** 9 March 2017 **Published** 27 April 2017

Citation Falida K, Eilebrecht S, Gunst K, zur Hausen H, de Villiers E-M. 2017. Isolation of two virus-like circular DNAs from commercially available milk samples. *Genome Announc* 5: e00266-17. <https://doi.org/10.1128/genomeA.00266-17>.

Copyright © 2017 Falida 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 Ethel-Michele de Villiers, e.devilliers@dkfz.de.

REFERENCES

1. zur Hausen H, de Villiers EM. 2015. Dairy cattle serum and milk factors contributing to the risk of colon and breast cancers. *Int J Cancer* 137: 959–967. <https://doi.org/10.1002/ijc.29466>.
2. Butcher J. 1976. The distribution of multiple sclerosis in relation to the dairy industry and milk consumption. *N Z Med J* 83:427–430.
3. Whitley C, Gunst K, Müller H, Funk M, Zur Hausen H, de Villiers EM. 2014. Novel replication-competent circular DNA molecules from healthy cattle serum and milk and multiple sclerosis-affected human brain tissue. *Genome Announc* 2:e00849-14. <https://doi.org/10.1128/genomeA.00849-14>.
4. Lamberto I, Gunst K, Müller H, Zur Hausen H, de Villiers EM. 2014. Mycovirus-like DNA virus sequences from cattle serum and human brain and serum samples from multiple sclerosis patients. *Genome Announc* 2(4):e00848-14. <https://doi.org/10.1128/genomeA.00848-14>.
5. Gunst K, Zur Hausen H, de Villiers EM. 2014. Isolation of bacterial plasmid-related replication-associated circular DNA from a serum sample of a multiple sclerosis patient. *Genome Announc* 2(4):e00847-14. <https://doi.org/10.1128/genomeA.00847-14>.
6. Funk M, Gunst K, Lucansky V, Müller H, Zur Hausen H, de Villiers EM. 2014. Isolation of protein-associated circular DNA from healthy cattle serum. *Genome Announc* 2(4):e00846-14. <https://doi.org/10.1128/genomeA.00846-14>.