Complete Genome Sequence of *Carnobacterium* sp. 17-4[▽]

Sonja Voget,² Barbara Klippel,³ Rolf Daniel,^{1,2*} and Garabed Antranikian³

Department of Genomic and Applied Microbiology¹ and Göttingen Genomics Laboratory,² Institute of Microbiology and Genetics, Georg-August University Göttingen, Grisebachstr. 8, 37077 Göttingen, Germany, and Institute of Technical Microbiology, Hamburg University of Technology, Kasernenstr. 12, 21073 Hamburg, Germany³

Received 18 April 2011/Accepted 24 April 2011

Members of the carnobacteria have been extensively studied as probiotic cultures in aquacultures and protective cultures in seafood, dairy, and meat. We report on the finished genome sequence of *Carnobacterium* sp. 17-4, which has been isolated from permanently cold seawater. The genetic information reveals a new circular bacteriocin biosynthesis cluster.

Carnobacteria are ubiquitous lactic acid bacteria, which have been frequently isolated from temperate to polar aquatic environments and food products such as seafood, dairy, and meat (1, 4, 8, 11, 12). *Carnobacterium* sp. 17-4 was isolated from a seawater sample taken at the Isfjord, Spitsbergen, in autumn 1999 (7). Analysis of the 16S rRNA gene showed that *Carnobacterium* sp. 17-4 is closely related to the protease-producing arctic isolate *Carnobacterium* sp. ARCTIC-P35.

The genome sequence of *Carnobacterium* sp. 17-4 was determined by using a 454 GS-FLX system (Roche 454 Life Science, Mannheim, Germany). Pyrosequencing runs, including two shotgun runs and one paired-end run, resulted in 37-fold coverage. The initial assembly yielded 19 scaffolds containing 69 contigs. PCR-based techniques and Sanger sequencing of the products were used to close the remaining gaps. The manually curated and annotated final sequence of *Carnobacterium* sp. 17-4 comprises one chromosome of 2,635,294 bp and one plasmid of 50,105 bp. The chromosome (35.25% G+C content) comprises 2,420 predicted protein-encoding genes, 67 tRNA genes, 8 rRNA operons, and 1 single 5S rRNA gene. The plasmid (31.53% G+C content) harbors 54 protein-encoding genes.

Whereas complete genome sequences from many lactic acid bacteria are available, only one draft genome sequence from a member of the genus *Carnobacterium* (*Carnobacterium* sp. AT7) has been previously deposited in the databases. *Carnobacterium* sp. AT7 is a piezophilic strain isolated from the Aleutian trench (9). We used the draft data set of this organism for comparisons. To identify orthologous genes and functions as well as strain-specific gene content, a combination of bidirectional BLAST and a global sequence alignment was employed. Overall, *Carnobacterium* sp. 17-4 and *Carnobacterium* sp. AT7 share 1,816 (74.70%) orthologous genes (E value [expect value] < 1e−20; similarity score > 30%). Among the 25.3% species-specific genes of *Carnobacterium* sp. 17-4, 18.5% belong to the COG categories for transport and metabolism

(G, E, F, H, and I) and more precisely to the subcategory carbohydrate transport and metabolism.

Only the replication-associated proteins encoded by the plasmid of *Carnobacterium* sp. 17-4 shared homologies with putative gene products encoded by a 76,048 bp-contig of *Carnobacterium* sp. AT7, indicating that this putative plasmid and the plasmid of *Carnobacterium* sp. 17-4 differ in content and size. In addition to the high number of carbohydrate transport and metabolism genes found on the chromosome, three putative carbohydrate phosphotransferase systems (PTS) are encoded by the plasmid of *Carnobacterium* sp. 17-4.

A new circular bacteriocin biosynthesis cluster was located in a genomic island of *Carnobacterium* sp. 17-4. Bacteriocins are antimicrobial peptides that are active against closely related strains. Bacteriocins produced by lactic acid bacteria are generally regarded as safe and possess a high biotechnological potential for various applications such as food preservation and probiotics (1, 2, 3, 5). The genetic organization of the new cluster is similar to that of a reported circular bacteriocin, carnocyclin A, which is produced by *Carnobacterium maltaromaticum* UAL307 (6, 10). In contrast to the genomic synteny, only little sequence homology was observed.

Nucleotide sequence accession numbers. The complete genome and plasmid sequences of *Carnobacterium* sp. 17-4 have been deposited in GenBank under accession numbers CP002563 and CP002564, respectively.

We thank Frauke D. Meyer for providing excellent technical assistance and Rüdiger Lehmann for bioinformatics support.

This work was funded by the GenoMikPlus initiative of the German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung [BMBF]).

REFERENCES

1. Afzal, M. I., et al. 2010. *Carnobacterium maltaromaticum*: identification, isolation tools, ecology and technological aspects in dairy products. *Food Microbiol.* **27**:573–579.
2. Avonts, L., E. V. Uytven, and L. D. Vuyst. 2004. Cell growth and bacteriocin production of probiotic *Lactobacillus* strains in different media. *Int. Dairy J.* **14**:947–955.
3. De Vuyst, L., and F. Leroy. 2007. Bacteriocins from lactic acid bacteria: production, purification, and food applications. *J. Mol. Microbiol. Biotechnol.* **13**:194–199.
4. Galkin, A., L. Kulakova, H. Ashida, Y. Sawa, and N. Esaki. 1999. Cold-adapted alanine dehydrogenases from two Antarctic bacterial strains: gene cloning, protein characterization, and comparison with mesophilic and thermophilic counterparts. *Appl. Environ. Microbiol.* **65**:4014–4020.

* Corresponding author. Mailing address: Department of Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, Georg-August University Göttingen, Grisebachstr. 8, D-37077 Göttingen, Germany. Phone: 49-551-393827. Fax: 49-551-3912181. E-mail: rdaniel@gwdg.de.

[▽] Published ahead of print on 6 May 2011.

5. **Gálvez, A., H. Abriouel, R. L. López, and N. B. Omar.** 2007. Bacteriocin-based strategies for food biopreservation. *Int. J. Food Microbiol.* **120**:51–70.
6. **Gong, X., L. A. Martin-Visscher, D. Nahirney, J. C. Vederas, and M. Duszyn.** 2009. The circular bacteriocin, carnocyclin A, forms anion-selective channels in lipid bilayers. *Biochim. Biophys. Acta* **1788**:1797–1803.
7. **Groudieva, T., et al.** 2004. Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. *Extremophiles* **8**:475–488.
8. **Holley, R. A., T. Y. Guan, M. Y. Peirson, and C. K. Yost.** 2002. *Carnobacterium viridans* sp. nov., an alkaliphilic, facultative anaerobe isolated from refrigerated, vacuum-packed bologna sausage. *Int. J. Syst. Evol. Microbiol.* **52**:1881–1885.
9. **Lauro, F. M., R. A. Chastain, L. E. Blankenship, A. A. Yayanos, and D. H. Bartlett.** 2007. The unique 16S rRNA genes of piezophiles reflect both phylogeny and adaptation. *Appl. Environ. Microbiol.* **73**:838–845.
10. **Martin-Visscher, L. A., et al.** 2008. Isolation and characterization of carnocyclin A, a novel circular bacteriocin produced by *Carnobacterium maltaromaticum* UAL307. *Appl. Environ. Microbiol.* **74**:4756–4763.
11. **Matamoros, S., M. F. Pilet, F. Gigout, H. Prévost, and F. Leroi.** 2009. Selection and evaluation of seafood-borne psychrotrophic lactic acid bacteria as inhibitors of pathogenic and spoilage bacteria. *Food Microbiol.* **26**:638–644.
12. **Yanagida, F., Y. S. Chen, and M. Yasaki.** 2007. Isolation and characterization of lactic acid bacteria from lakes. *J. Basic Microbiol.* **47**:184–190.