Journal of Bacteriology

Complete Genome Sequence of Acidaminococcus intestini RYC-MR95, a Gram-Negative Bacterium from the Phylum Firmicutes

Giuseppe D'Auria, Juan-Carlos Galán, Manuel Rodríguez-Alcayna, Andrés Moya, Fernando Baquero and Amparo Latorre

J. Bacteriol. **2011, 193(24):7008. DOI: 10.1128/JB.06301-11.**

JOURNAL OF BACTERIOLOGY, Dec. 2011, p. 7008–7009 Vol. 193, No. 24
0021-9193/11/$12.00 doi:10.1128/JB.06301-11

Complete Genome Sequence of *Acidaminococcus intestini* RYC-MR95, a Gram-Negative Bacterium from the Phylum *Firmicutes*

Giuseppe D'Auria,[1,2]* Juan-Carlos Galán,[2,3,4] Manuel Rodríguez-Alcayna,[3] Andrés Moya,[1,2] Fernando Baquero,[2,3,4] and Amparo Latorre[1,2]

Joint Unit of Research in Genomics and Health, Centre for Public Health Research (CSISP)-Cavanilles Institute for Biodiversity and Evolutionary Biology (University of Valencia), Valencia, Spain[1]; Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública (CIBERESP), Madrid, Spain[2]; Servicio de Microbiología, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain[3]; and Unidad de Resistencia a Antibióticos y Virulencia Bacteriana (RYC-CSIC), Madrid, Spain[4]

Received 4 October 2011/Accepted 10 October 2011

***Acidaminococcus intestini* belongs to the family *Acidaminococcaceae*, order *Selenomonadales*, class *Negativicutes*, phylum *Firmicutes*. *Negativicutes* show the double-membrane system of Gram-negative bacteria, although their chromosomal backbone is closely related to that of Gram-positive bacteria of the phylum *Firmicutes*. The complete genome of a clinical *A. intestini* strain is here presented.**

In 2007, *Acidaminococcus intestini* was defined as a new species belonging to the family *Acidaminococcaceae* (9). Its taxonomic location is controversial, with various taxonomical reassignments occurring. Initially, *Acidaminococcus* was included as a member of the family *Neisseriaceae* according to its phenotypic characteristics (12). In 1984, anaerobic Gram-negative cocci such as *Acidaminococcus* were transferred from *Neisseriaceae* to the *Veillonellaceae* family (13). Based on the 16S rRNA gene sequence, *Acidaminococcus* was reclassified as belonging to cluster IX in the class *Clostridia* in the phylum *Firmicutes* (5). Since then, this cluster has been continuously enriched with novel species (6). Recently, a new class has been proposed within the phylum *Firmicutes*, named *Negativicutes* and containing all genera with a Gram-negative cell wall, including the family *Acidaminococcaceae* (10). It has been suggested that the class *Negativicutes* could have resulted from a different evolutionary trajectory than that of traditional Gram-negative bacteria, giving more interest to assessing the complete genome of members of this class (8).

A. intestini is known to be a normal commensal of the human gut, representing ~1% of the fecal bacterial population (2). It has been occasionally related to infective processes but always associated with polymicrobial infections (3, 6, 11) and might act as a reservoir of antibiotic resistance mechanisms (7). The *A. intestini* RYC-MR95 strain was isolated from a perianal abscess of a diabetic patient. It was originally grown on brucella agar plates supplemented with hemin and vitamin K1 and incubated at 37°C in an anaerobic atmosphere. The identification was performed according to the detection of accumulated butyric acid using gas-liquid chromatography and sequencing of the 16S rRNA gene.

The genome was sequenced using the 454 GS FLX system. The starting data set before assembly contained about 400,000 GS FLX single reads and a half 3-kb paired-end run which produced about 390,000 sequences, of which about 164,000 were made by paired-end couples. All reads were assembled *de novo* by MIRA software (4). Assembly was completed manually with the use of Gap4 software from the Staden package (14). Finally, repeated and dubious regions were confirmed by Sanger resequencing.

The chromosomal size was 2,488,745 bp; 2,167 open reading frames (ORFs) were annotated covering 86.7% of the genome length; 52 tRNAs covering all bacterial amino acids and three ribosomal operons were annotated. Small ribosomal proteins RP-S1 to RP-S19 and large ribosomal proteins RP-L1 to RP-L35 and RP-L7A were also found.

A bacteriophage resistance system, CRISPR (1), was also identified; it comprised 52 repeats as well as at least two CRISPR-associated proteins.

Nucleotide sequence accession number. The genome was deposited in GenBank under accession number CP003058.

This work has been funded by projects Prometeo/2009/092 and GVPRE/2008/099 from Generalitát Valenciana; project SAF2009-13032-C02-01 from the Ministerio de Ciencia e Innovación, Spain; and projects PAR-241476 LSHM-CT-2005-518152 and LSHM-CT-2005-018705 from the European Community.

* Corresponding author. Mailing address: Joint Unit of Research in Genomics and Health, Centre for Public Health Research (CSISP)-Cavanilles Institute for Biodiversity and Evolutionary Biology (University of Valencia), Valencia, Spain. Phone: 34961925929. Fax: 34961925703. E-mail: dauria_giu@gva.es.

REFERENCES

1. **Barrangou, R., et al.** 2007. CRISPR provides acquired resistance against viruses in prokaryotes. Science **315:**1709–1712.
2. **Callaway, T. R., et al.** 2010. Evaluation of bacterial diversity in the rumen and feces of cattle fed different levels of dried distillers grains plus solubles using bacterial tag-encoded FLX amplicon pyrosequencing. J. Anim. Sci. **88:**3977–3983.
3. **Chatterjee, B. D., and C. K. Chakraborti.** 1995. Non-sporing anaerobes in certain surgical group of patients. J. Indian Med. Assoc. **93:**333–339.
4. **Chevreux, B., T. Pfisterer, T. Wetter, and S. Suhai.** 1999. Genome sequence assembly using trace signals and additional sequence information, p. 45–56. *In* Proceedings of the German Conference on Bioinformatics (GCB) 1999. Deutsches Krebsforschungszentrum, Heidelberg, Germany.
5. **Collins, M. D., et al.** 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int. J. Syst. Bacteriol. **44:**812–826.
6. **De Vos, P., et al.** 2009. Bergey's manual of systematic bacteriology, vol. 3. The Firmicutes. Springer, New York, NY.
7. **Galán, J. C., M. Reig, A. Navas, F. Baquero, and J. Blázquez.** 2000. ACI-1

from *Acidaminococcus fermentans*: characterization of the first beta-lactamase in anaerobic cocci. Antimicrob. Agents Chemother. **44:**3144–3149.
8. **Gupta, R. S.** 2011. Origin of diderm (Gram-negative) bacteria: antibiotic selection pressure rather than endosymbiosis likely led to the evolution of bacterial cells with two membranes. Antonie Van Leeuwenhoek **100:**171–182.
9. **Jumas-Bilak, E., et al.** 2007. *Acidaminococcus intestini* sp. nov., isolated from human clinical samples. Int. J. Syst. Evol. Microbiol. **57:**2314–2319.
10. **Marchandin, H., et al.** 2010. *Negativicoccus succinicivorans* gen. nov., sp. nov., isolated from human clinical samples, emended description of the family Veillonellaceae and description of Negativicutes classis nov., Selenomonadales ord. nov. and Acidaminococcaceae fam. nov. in the bacterial phylum Firmicutes. Int. J. Syst. Evol. Microbiol. **60:**1271–1279.
11. **Peraino, V. A., S. A. Cross, and E. J. Goldstein.** 1993. Incidence and clinical significance of anaerobic bacteriemia in a community hospital. Clin. Infect. Dis. **16**(Suppl. 4)**:**S288–S291.
12. **Rogosa, M.** 1969. *Acidaminococcus* gen. n., *Acidaminococcus fermentans* sp.n., anaerobic Gram-negative diplococcus using amino acids as the sole energy source for growth. J. Bacteriol. **98:**756–766.
13. **Rogosa, M.** 1984. Anaerobic Gram-negative cocci, p. 680–685. *In* J. G. Holt et al. (ed.), Bergey's manual of systematic bacteriology, vol. 1. Williams & Wilkins, Baltimore, MD.
14. **Staden, R.** 1996. The Staden sequence analysis package. Mol. Biotechnol. **5:**233–241.