



Genome Sequence of the Bile Salt-Degrading Bacterium *Novosphingobium* sp. Strain Chol11, a Model Organism for Bacterial Steroid Catabolism

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ABSTRACT Many bacteria from different phylogenetic groups are able to degrade eukaryotic steroid compounds, but the underlying metabolic pathways are still not well understood. *Novosphingobium* sp. strain Chol11 is a steroid-degrading alphaproteobacterium. Its genome sequence reveals that it lacks several genes for steroid degradation known to exist in other model organisms.

Be.g., in digestion of lipophilic nutrients in the intestinal tract (1, 2). These steroids are released by fecal and urinal excretion into the environment, where they can serve as a source of carbon and energy for many soil and aquatic bacteria (3, 4). Bile salt degradation has been studied mainly with *Pseudomonas stutzeri* strain Chol1, *Comamonas testosteroni, Rhodococcus jostii*, and *Pseudomonas putida* (5–8) and involves very similar metabolic pathways in all of these model organisms. However, some bacteria were shown to initiate the degradation of 7α -hydroxy-bile salts by an alternative pathway (3). Among these is the alphaproteobacterium *Novosphingobium* sp. strain Chol11, which has been isolated from freshwater. Degradation of the bile salt cholate is initiated by the 7α -dehydratase Hsh2 leading to metabolites with a 3-keto- $\Delta^{4,6}$ -diene structure of the steroid skeleton (9). The steroid skeleton of these metabolites cannot be degraded by, e.g., *P. stutzeri* strain Chol1 (4, 9). For further investigating this potential novel pathway for bile salt degradation, the genome of *Novosphingobium* sp. strain Chol11 was sequenced.

Genomic DNA was purified with the Puregene tissue core kit B (Qiagen). Purified DNA was used to construct an 8-kbp mate pair sequencing library (Illumina, USA). Assembly of the obtained and processed reads was performed by applying GS *de novo* Assembler software version 2.8 (Roche, Mannheim, Germany). Assembly resulted in 52 contigs and 10 scaffolds. *Novosphingobium* sp. strain Chol11 scaffolds were mapped onto the *Sphingobium chloroplenolium* L-1 (GenBank accession no. CP002798 to CP002800, BioProject no. PRJNA224116) reference replicons by means of r2cat (10), followed by an *in silico* gap closure approach (11). Annotation of the draft replicons was performed within the GenDB2.0 system (12) and Prokka version 1.11 (13).

The Chol11 draft genome comprises two chromosomes and two plasmids. Chromosome 1 (2.54 Mb) and chromosome 2 (0.86 Mb) encode 2,494 and 776 open reading frames (ORFs), respectively. Together with the plasmids pSa (circular, ca. 0.13 Mb) and pSb (linear, 0.13 Mb), the total genome size is about 3.66 Mb and encodes 3,532 putative proteins. The mean GC content is 62.44%.

Most of the genes with a potential role in steroid degradation are located on chromosome 2, including *hsh2*. Compared to the genome of, e.g., *Pseudomonas stutzeri* Chol1 DSM 103613 (14), several genes involved in the degradation of the steroid side chain do not have orthologues in strain Chol11. Thus, the genomic content supports

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the presence of an unexplored pathway for bile salt degradation in *Novosphingobium* sp. strain Chol11.

Accession number(s). The scaffolds of the whole-genome shotgun project have been deposited in the EMBL database (EBI) under the accession no. OBMU01000001 to OBMU01000010.

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REFERENCES

- 1. Hofmann AF, Mysels KJ. 1987. Bile-salts as biological surfactants. Colloids Surf 30:145–173. https://doi.org/10.1016/0166-6622(87)80207-X.
- Hylemon PB, Zhou H, Pandak WM, Ren S, Gil G, Dent P. 2009. Bile acids as regulatory molecules. J Lipid Res 50:1509–1520. https://doi.org/10 .1194/jlr.R900007-JLR200.
- Philipp B. 2011. Bacterial degradation of bile salts. Appl Microbiol Biotechnol 89:903–915. https://doi.org/10.1007/s00253-010-2998-0.
- Holert J, Yücel O, Suvekbala V, Kulić Z, Möller H, Philipp B. 2014. Evidence of distinct pathways for bacterial degradation of the steroid compound cholate suggests the potential for metabolic interactions by interspecies cross-feeding. Environ Microbiol 16:1424–1440. https://doi.org/10.1111/ 1462-2920.12407.
- Barrientos Á, Merino E, Casabon I, Rodríguez J, Crowe AM, Holert J, Philipp B, Eltis LD, Olivera ER, Luengo JM. 2015. Functional analyses of three acyl-CoA synthetases involved in bile acid degradation in *Pseudomonas putida*DOC21. Environ Microbiol 17:47–63. https://doi.org/10 .1111/1462-2920.12395.
- Holert J, Yücel O, Jagmann N, Prestel A, Möller HM, Philipp B. 2016. Identification of bypass reactions leading to the formation of one central steroid degradation intermediate in metabolism of different bile salts in *Pseudomonas* sp. strain Chol1. Environ Microbiol 18:3373–3389. https:// doi.org/10.1111/1462-2920.13192.
- Horinouchi M, Hayashi T, Kudo T. 2012. Steroid degradation in *Comamonas testosteroni*. J Steroid Biochem Mol Biol 129:4–14. https://doi.org/ 10.1016/j.jsbmb.2010.10.008.
- 8. Swain K, Casabon I, Eltis LD, Mohn WW. 2012. Two transporters essential for reassimilation of novel cholate metabolites by *Rhodococcus*

jostii RHA1. J Bacteriol 194:6720-6727. https://doi.org/10.1128/JB .01167-12.

- Yücel O, Drees S, Jagmann N, Patschkowski T, Philipp B. 2016. An unexplored pathway for degradation of cholate requires a 7α-hydroxysteroid dehydratase and contributes to a broad metabolic repertoire for the utilization of bile salts in *Novosphingobium* sp. strain Chol11. Environ Microbiol 18:5187–5203. https://doi.org/10.1111/1462-2920.13534.
- Husemann P, Stoye J. 2010. r2cat: synteny plots and comparative assembly. Bioinformatics 26:570–571. https://doi.org/10.1093/bioinformatics/ btp690.
- 11. Wibberg D, Blom J, Jaenicke S, Kollin F, Rupp O, Scharf B, Schneiker-Bekel S, Sczcepanowski R, Goesmann A, Setubal JC, Schmitt R, Pühler A, Schlüter A. 2011. Complete genome sequencing of *Agrobacterium* sp. H13-3, the former *Rhizobium lupini* H13-3, reveals a tripartite genome consisting of a circular and a linear chromosome and an accessory plasmid but lacking a tumor-inducing Ti-plasmid. J Biotechnol 155: 50–62. https://doi.org/10.1016/j.jbiotec.2011.01.010.
- Meyer F, Goesmann A, McHardy AC, Bartels D, Bekel T, Clausen J, Kalinowski J, Linke B, Rupp O, Giegerich R, Pühler A. 2003. GenDB—an open source genome annotation system for prokaryote genomes. Nucleic Acids Res 31:2187–2195. https://doi.org/10.1093/nar/gkg312.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
- Holert J, Alam I, Larsen M, Antunes A, Bajic VB, Stingl U, Philipp B. 2013. Genome sequence of *Pseudomonas* sp. strain Chol1, a model organism for the degradation of bile salts and other steroid compounds. Genome Announc 1:e00014-12. https://doi.org/10.1128/genomeA.00014-12.