



HAL
open science

Genome Sequences of Two Non domesticated *Bacillus subtilis* Strains Able To Form Thick Biofilms on Submerged Surfaces

P. Sanchez-Vizueté, K. Tanaka, A. Bridier, Y. Shirae, K.-I. Yoshida, T. Bouchez, S. Aymerich, Romain Briandet, D. Le Coq

► To cite this version:

P. Sanchez-Vizueté, K. Tanaka, A. Bridier, Y. Shirae, K.-I. Yoshida, et al.. Genome Sequences of Two Non domesticated *Bacillus subtilis* Strains Able To Form Thick Biofilms on Submerged Surfaces. *Genome Announcements*, 2014, 2 (5), 10.1128/genomeA.00946-14 . hal-03031307

HAL Id: hal-03031307

<https://hal.inrae.fr/hal-03031307>

Submitted on 25 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Genome Sequences of Two Nondomesticated *Bacillus subtilis* Strains Able To Form Thick Biofilms on Submerged Surfaces

Pilar Sanchez-Vizueté,^{a,b} Kosei Tanaka,^c Arnaud Bridier,^d Yusuke Shirae,^e Ken-ichi Yoshida,^e Théodore Bouchez,^d Stéphane Aymerich,^{a,b} Romain Briandet,^{a,b} Dominique Le Coq^{a,b,f}

INRA, UMR1319 Micalis, Jouy-en-Josas, France^a; AgroParisTech, UMR Micalis, Jouy-en-Josas, France^b; Organization of Advanced Science and Technology, Kobe University, Kobe, Japan^c; IRSTEA, UR HBAN, Antony, France^d; Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University, Kobe, Japan^e; CNRS, Jouy-en-Josas, France^f

P.S.-V. and K.T. contributed equally to this work.

Genomes of two nondomesticated strains of *Bacillus subtilis* subspecies *subtilis*, NDmed and NDfood, have been sequenced. Both strains form very thick and spatially complex biofilms on submerged surfaces. Moreover, biofilms of the NDmed isolate were shown to be highly resistant to antimicrobials action.

Received 20 August 2014 Accepted 26 August 2014 Published 25 September 2014

Citation Sanchez-Vizueté P, Tanaka K, Bridier A, Shirae Y, Yoshida K-I, Bouchez T, Aymerich S, Briandet R, Le Coq D. 2014. Genome sequences of two nondomesticated *Bacillus subtilis* strains able to form thick biofilms on submerged surfaces. *Genome Announc.* 2(5):00946-14. doi:10.1128/genomeA.00946-14.

Copyright © 2014 Sanchez-Vizueté et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Dominique Le Coq, dominique.le-coq@grignon.inra.fr.

Bacillus subtilis is a non-pathogenic Gram-positive bacterium largely used in biotechnological processes and academic research. Although this bacterium is mainly found in the soil, several strains have recently been isolated from other environments (1–6). *B. subtilis* is able to form structured biofilms in which cells are embedded in a self-produced matrix of polymers. For studying biofilm properties and the associated genetic regulation, most researchers have used the “less domesticated” strain NCIB3610 instead of the reference strain 168, which is defective for determinants involved in multicellular behavior (7). Here we present the genome sequences of two *B. subtilis* natural isolates. The first strain, NDmed, was isolated from an endoscope washer disinfectant in a hospital in England (8). The second strain, NDfood, was isolated from a dairy product in France (9). Both strains are able to form spatially organized biofilms with protruding structures on submerged surfaces as well as wrinkled macrocolonies and robust pellicles (9). In addition, submerged biofilms of NDmed exhibited high resistance to antimicrobials and the ability to protect pathogens (10).

Genome sequencing of *B. subtilis* NDmed and NDfood strains was performed using Illumina Miseq technology. A total of 4,595,294 and 3,622,152 reads were obtained for NDmed and NDfood, respectively. The raw sequences generated were mapped to the reference strain 168 genome (11) and using the CLC NGS assembler, the reads were assembled into 10 contigs for NDmed (ranging from 4.9 kb to 1.2 Mb) and 12 contigs for NDfood (ranging from 1.4 kb to 1.1 Mb), with an average coverage of 271-fold and 214-fold, respectively. For both strains the total size of the assembly was around 4.06 Mb and the G+C content about 43.7%.

Comparison of NDmed and NDfood sequences to the sequence of the reference strain 168 established that these two nondomesticated isolates are very close to the reference strain 168, with less than 100 single-nucleotide polymorphisms (SNPs) and

less than 50 insertions/deletions, and among which the great majority were common to both strains.

As in other *B. subtilis* natural isolates (e.g., BsP1 [1], RO-NN-1 [4], BsN5 [12]), the SPβ prophage (134.4 kb) and the conjugative element ICEBs1 (20.5 kb) are missing in NDmed and NDfood strains. However, a putative prophage (44.2 kb) is present in both strains immediately downstream of the *glnA* gene. A highly similar prophage can be found at the same position in strains AUSI98 (4) and PS216 (5). No plasmids were observed in NDmed and NDfood strains, such as the one present in NCIB3610 which encodes the ComI transformation inhibitor (13). Interestingly, the two nondomesticated strains presented here are naturally competent, which facilitates their use in research.

The genomes of NDmed and NDfood isolates could provide a better understanding of *B. subtilis* social behavior in natural environments. Although all the biofilm genes found defective in strain 168 have an identical sequence in NDmed, NDfood, and NCIB3610, other differences found between this “less domesticated” strain and the two nondomesticated isolates would shed light to their specific phenotypes.

Nucleotide sequence accession numbers. These two whole-genome shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers JPVW00000000 for *B. subtilis* NDmed and JPVX00000000 for *B. subtilis* NDfood. The versions described in this paper are the first versions, JPVW01000000 and JPVX01000000.

ACKNOWLEDGMENTS

We thank C. Neuveglise for helpful advices and fruitful discussions.

P. Sanchez-Vizueté is the recipient of a Ph.D. grant from the Région Île-de-France (DIM ASTREA). This work was supported by Special Coordination Funds for Creation of Innovative Centers for Advanced Interdisciplinary Research Areas from JST, MEXT (Japan), and by INRA funding (France).

REFERENCES

- Schyns G, Serra CR, Lapointe T, Pereira-Leal JB, Potot S, Fickers P, Perkins JB, Wyss M, Henriques AO. 2013. Genome of a gut strain of *Bacillus subtilis*. *Genome Announc.* 1(1):e00184-12. <http://dx.doi.org/10.1128/genomeA.00184-12>.
- Matulová M, Husárová S, Capek P, Sancelme M, Delort A-M. 2011. NMR structural study of fructans produced by *Bacillus* sp. 3B6, bacterium isolated in cloud water. *Carbohydr. Res.* 346:501–507. <http://dx.doi.org/10.1016/j.carres.2010.12.012>.
- Karlyshev AV, Melnikov VG, Chikindas ML. 2014. Draft genome sequence of *Bacillus subtilis* strain KATMIRA1933. *Genome Announc.* 2(3):e00619-14. <http://dx.doi.org/10.1128/genomeA.00619-14>.
- Earl AM, Eppinger M, Fricke WF, Rosovitz MJ, Rasko DA, Daugherty S, Losick R, Kolter R, Ravel J. 2012. Whole-genome sequences of *Bacillus subtilis* and close relatives. *J. Bacteriol.* 194:2378–2379. <http://dx.doi.org/10.1128/JB.05675-11>.
- Durrett R, Miras M, Mirouze N, Narechania A, Mandic-Mulec I, Dubnau D. 2013. Genome sequence of the *Bacillus subtilis* biofilm-forming transformable strain PS216. *Genome Announc.* 1(3):e00288-13. <http://dx.doi.org/10.1128/genomeA.00288-13>.
- Chen Y, Yan F, Chai Y, Liu H, Kolter R, Losick R, Guo JH. 2013. Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. *Environ. Microbiol.* 15:848–864. <http://dx.doi.org/10.1111/j.1462-2920.2012.02860.x>.
- McLoon AL, Guttenplan SB, Kearns DB, Kolter R, Losick R. 2011. Tracing the domestication of a biofilm-forming bacterium. *J. Bacteriol.* 193:2027–2034. <http://dx.doi.org/10.1128/JB.01542-10>.
- Martin DJH, Denyer SP, McDonnell G, Maillard J-Y. 2008. Resistance and cross-resistance to oxidising agents of bacterial isolates from endoscope washer disinfectors. *J. Hosp. Infect.* 69:377–383. <http://dx.doi.org/10.1016/j.jhin.2008.04.010>.
- Bridier A, Le Coq D, Dubois-Brissonnet F, Thomas V, Aymerich S, Briandet R. 2011. The spatial architecture of *Bacillus subtilis* biofilms deciphered using a surface-associated model and *in situ* imaging. *PLoS One* 6:e16177. <http://dx.doi.org/10.1371/journal.pone.0016177>.
- Bridier A, Sanchez-Vizuet Mdelp, Le Coq D, Aymerich S, Meylheuc T, Maillard JY, Thomas V, Dubois-Brissonnet F, Briandet R. 2012. Biofilms of a *Bacillus subtilis* Hospital Isolate Protect *Staphylococcus aureus* from biocide Action. *PLoS One* 7:e44506. <http://dx.doi.org/10.1371/journal.pone.0044506>.
- Kunst F, Ogasawara N, Moszer I, Albertini AM, Alloni G, Azevedo V, Bertero MG, Bessières P, Bolotin A, Borchert S, Borriss R, Boursier L, Brans A, Braun M, Brignell SC, Bron S, Brouillet S, Bruschi CV, Caldwell B, Capuano V, Carter NM, Choi SK, Cordani JJ, Connerton IF, Cummings NJ, Daniel RA, Denzot F, Devine KM, Dusterhöft A, Ehrlich SD, Emmerson PT, Entian KD, Errington J, Fabret C, Ferrari E, Foulger D, Fritz C, Fujita M, Fujita Y, Fuma S, Galizzi A, Galleron N, Ghim SY, Glaser P, Goffeau A, Golightly EJ, Grandi G, Guiseppe G, Guy BJ, Haga K, Haiech J, Harwood CR, Hénaut A, Hilbert H, Holsappel S, Hosono S, Hullo MF, Itaya M, Jones L, Joris B, Karamata D, Kasahara Y, Klaerr-Blanchard M, Klein C, Kobayashi Y, Koetter P, Koningstein G, Krogh S, Kumano M, Kurita K, Lapidus A, Lardinois S, Lauber J, Lazarevic V, Lee SM, Levine A, Liu H, Masuda S, Mauël C, Médigue C, Medina N, Mellado RP, Mizuno M, Moestl D, Nakai S, Noback M, Noone D, O'Reilly M, Ogawa K, Ogiwara A, Oudega B, Park SH, Parro V, Pohl TM, Portelle D, Porwollik S, Prescott AM, Presecan E, Pujic P, Purnelle B, Rapoport G, Rey M, Reynolds S, Rieger M, Rivolta C, Rocha E, Roche B, Rose M, Sadaie Y, Sato T, Scanlan E, Schleich S, Schroeter R, Scoffone F, Sekiguchi J, Sekowska A, Seror SJ, Serror P, Shin BS, Soldo B, Sorokin A, Tacconi E, Takagi T, Takahashi H, Takemaru K, Takeuchi M, Tamakoshi A, Tanaka T, Terpstra P, Togoni A, Tosato V, Uchiyama S, Vandebol M, Vannier F, Vassarotti A, Viari A, Wambutt R, Wedler H, Weitzenegger T, Winters P, Wipat A, Yamamoto H, Yamane K, Yasumoto K, Yata K, Yoshida K, Yoshikawa HF, Zumstein E, Yoshikawa H, Danchin A. 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature* 390:249–256. <http://dx.doi.org/10.1038/36786>.
- Deng Y, Zhu Y, Wang P, Zhu L, Zheng J, Li R, Ruan L, Peng D, Sun M. 2011. Complete genome sequence of *Bacillus subtilis* BSn5, an endophytic bacterium of *Amorphophallus konjac* with antimicrobial activity for the plant pathogen *Erwinia carotovora* subsp. *carotovora*. *J. Bacteriol.* 193:2070–2071. <http://dx.doi.org/10.1128/JB.00129-11>.
- Konkol MA, Blair KM, Kearns DB. 2013. Plasmid-encoded ComI inhibits competence in the ancestral 3610 strain of *Bacillus subtilis*. *J. Bacteriol.* 195:4085–4093. <http://dx.doi.org/10.1128/JB.00696-13>.