



Draft Genome Sequences of Eight Bacilli Isolated from an Ancient Roman Amphora

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ABSTRACT Paleomicrobiology, the study of ancient microbiological material, allows us to understand different evolutionary phenomena in bacteria. In this study, eight bacilli isolated from an ancient Roman amphora, which dates to the IV to V sec. AD, were sequenced and functionally annotated.

Bacteria belonging to *Bacillus* spp. are capable of forming spores, specialized cell forms that can withstand adverse environmental conditions and extreme factors, such as temperature, radiation, and chemicals (1), also allowing for survival in a quiescent state for a long time (2). Isolation of these bacterial species from ancient sources has been previously reported, for example from a mummy (3) or ancient soil (4). In this study, bacilli from an ancient Roman amphora were isolated and sequenced. The amphora (IV to V century AD), found in Aquileia (UD-Italy) (45°45'05.9"N 13°21'03.8"E), was found intact and sealed with cementitious compound, thus preventing microbial contamination. It was opened under aseptic conditions in a laminar flow hood and GMP were followed to avoid contaminations. The inner material was sampled using 10 different culture media for bacteria and fungi by serial dilution method and enrichment steps. Growth (7.26 ± 0.09 log colony forming units/g) was observed only in brain heart infusion and plate count agar (Oxoid, Italy) after 48 h at 30°C under aerobic conditions, showing indented, diffuse mucosal colonies, 1 to 2.5 cm in diameter after 48 h at 30°C. The environmental control made using active/passive methods confirmed the absence of *Bacillus* spp. in the laboratory air. Twenty-five colonies present on the counting plates were isolated and examined for their morphological characteristics, which were Gram- and catalase-positive. Preliminary identification was performed by sequencing amplicons obtained using primers P1 and P4 (5), targeting V1 to V3 regions of 16S rDNA. Amplification conditions: final volume 50 μ L, 10 mM Tris-HCl, pH 8, KCl 50 mM, MgCl₂ 1.5 mM, dNTPs 0.2 mM, each primer 0.2 μ M, 1.25 U *Taq*-polymerase (Applied Biosystem, I), and 100 ng of DNA. After purification, products were sent to a commercial facility for sequencing (Sanger technology, Eurofins Genomics, Germany). Clones were eliminated by comparing genetic fingerprints (by RAPD, Rep-PCR, SAU-PCR) (6) of isolates and the resulting eight unique individual strains were subjected to whole-genome sequencing. For the sequencing process, each strain was cultured in brain heart infusion broth at 30°C for 48 h. After obtaining the cell pellet by centrifugation for 5 min at $5,000 \times g$, the DNA was extracted with the MagAttract HMW DNA Kit (Qiagen, Germany). The DNA was fragmented by sonication (BioRuptor-Diagenode, Belgium) and Celero DNA-Seq kit (Tecan, Swiss) was used for the preparation of libraries. The size of the individual fragments making up the library was measured using BioAnalyzer 2100 DNA chip electrophoresis (Agilent Technologies, USA) and sequencing was carried out with the MiSeq platform (Illumina, USA) in paired-end mode with reads of 300 bp length. The obtained. fastq files were analyzed and assembled using WGA-LP pipeline (7) with the following tools used in default mode. Raw reads were quality trimmed and deprived of Illumina adapters via Trimmomatic v0.39 (8). FastQC v0.11.9 (9) and Kraken2 v2.0.8-b (10)

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TABLE 1 Statistics of assembled genomes

| GeneBank accession no. | SRA accession no. | 16S RNA accession no. | Raw reads ^a | Strain | Organism name | Source | Coverage | Genome size ^b | Scaffolds ^b | N50 ^b | G+C content (%) ^c | CDS ^c | tRNAs ^c | Completeness (%) ^d |
|---------------------------|----------------------|--------------------------|---------------------------|----------|---------------------------|---------|----------|-----------------------------|------------------------|------------------|---------------------------------|------------------|--------------------|----------------------------------|
| JAKXEE000000000 | SRR18190504 | ON326590 | 2,727,696 | Aquil_B1 | <i>P. simplex</i> | Amphora | 234× | 5,649,653 | 25 | 910,271 | 40.2 | 5,402 | 81 | 98.91 |
| JAKXED000000000 | SRR18190503 | ON326591 | 717,606 | Aquil_B2 | <i>L. fusiformis</i> | Amphora | 75× | 4,643,302 | 34 | 1,011,198 | 37.5 | 4,546 | 85 | 99.93 |
| JAKXEC000000000 | SRR18190502 | ON326592 | 494,295 | Aquil_B3 | <i>B. muralis</i> | Amphora | 46× | 5,057,074 | 38 | 641,321 | 41.3 | 4,721 | 84 | 98.77 |
| JAKXEB000000000 | SRR18190501 | ON326593 | 1,480,745 | Aquil_B4 | <i>B. frigoritolerans</i> | Amphora | 65× | 6,677,279 | 68 | 317,977 | 39.5 | 6,548 | 92 | 98.91 |
| JAKXEA000000000 | SRR18190500 | ON326594 | 2,409,430 | Aquil_B5 | <i>B. muralis</i> | Amphora | 151× | 5,067,063 | 38 | 641,321 | 41.3 | 4,723 | 84 | 98.91 |
| JAKXDZ000000000 | SRR18190499 | ON326595 | 1,249,575 | Aquil_B6 | <i>P. psychrodurans</i> | Amphora | 108× | 4,256,356 | 79 | 253,084 | 35.9 | 4,213 | 70 | 100 |
| JAKXDY000000000 | SRR18190498 | ON326596 | 1,122,132 | Aquil_B7 | <i>B. frigoritolerans</i> | Amphora | 71× | 5,521,551 | 46 | 613,520 | 40.3 | 5,287 | 84 | 98.91 |
| JAKXDX000000000 | SRR18190497 | ON326597 | 1,742,298 | Aquil_B8 | <i>P. simplex</i> | Amphora | 57× | 5,654,249 | 78 | 193,610 | 40.2 | 5,437 | 81 | 98.91 |

^a Determined using FastQC.
^b Determined using Quast.
^c Determined using PGAP.
^d Determined using CheckM.

were used for quality and contamination control. Assembly was carried out using SPAdes v3.15.2 (11). The quality of the final assemblies was evaluated using CheckM v1.1.3 (12), Quast v5.0.2 (13), and SamTools v1.10 (14). Functional annotation was carried out on the genomes using PGAP 2022-04-14.build6021 (15).

Data availability. Sequences were deposited in GenBank with [PRJNA811801](#) BioProject accession number. Table 1 reports the GenBank and SRA accession number, the raw reads number, the NCBI taxonomic identification, the isolation source, the sequencing and assembly statistics, and the genome features of strains for each sample.

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REFERENCES

- Setlow P. 2006. Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *J Appl Microbiol* 101:514–525. <https://doi.org/10.1111/j.1365-2672.2005.02736.x>.
- Setlow P. 2014. Germination of spores of *Bacillus* species: what we know and do not know. *J Bacteriol* 196:1297–1305. <https://doi.org/10.1128/JB.01455-13>.
- Čavka M, Glasnović A, Janković I, Šikanjić PR, Perić B, Brkljačić B, Mlinarić-Missoni E, Škrlin J. 2010. Microbiological analysis of a mummy from the archeological museum in Zagreb. *Coll Antropol* 34:803–805.
- Southern PM. 2008. Archeology meets clinical microbiology: analysis of the microbial content of soil from archeological sites in Italy and Belize. *Lab Med* 39:601–602. <https://doi.org/10.1309/LMHJGYSYAPHNCNV9>.
- Klijn N, Weerkamp AH, De Vos WM. 1991. Identification of mesophilic lactic acid bacteria by using polymerase chain reaction-amplified variable regions of 16S rRNA and specific DNA probes. *Appl Environ Microbiol* 57:3390–3393. <https://doi.org/10.1128/aem.57.11.3390-3393.1991>.
- Iacumin L, Comi G, Cantoni C, Coccolin L. 2006. Molecular and technological characterization of *Staphylococcus xylosus* isolated from naturally fermented Italian sausages by RAPD, Rep-PCR and Sau-PCR analysis. *Meat Sci* 74:281–288. <https://doi.org/10.1016/j.meatsci.2006.03.020>.
- Rossi N, Colautti A, Iacumin L, Piazza C. 2021. WGA-LP: a pipeline for whole genome assembly of contaminated reads. *Bioinformatics* 38:846–848. <https://doi.org/10.1093/bioinformatics/btab719>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- Wood DE, Lu J, Langmead B. 2019. Improved metagenomic analysis with Kraken 2. *Genome Biol* 20:–13. <https://doi.org/10.1186/s13059-019-1891-0>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
- Zhao Y, Wu J, Yang J, Sun S, Xiao J, Yu J. 2012. PGAP: pan-genomes analysis pipeline. *Bioinformatics* 28:416–418. <https://doi.org/10.1093/bioinformatics/btr655>.