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Draft genome sequence of a hot spring bacterial isolate *Brevibacillus borstelensis* Gp-1 with potential extracellular lipase activity

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ABSTRACT

Our study is aimed to determine the draft genome of a novel strain of *Brevibacillus borstelensis* GP-1, a potential extracellular lipase producer, isolated from Ganeshpuri hot water springs (19.45 N, 72.79 E), from Maharashtra, India. It is a Gram-positive, long rod-shaped cell, with a central spore, gives fluorescent orange halos on Rhodamine B agar plates. Whole-genome sequence analysis using Illumina platform revealed that it is a novel strain with the closest relative being *Brevibacillus borstelensis* cifa_chp40. Whole-genome sequence assembly was deposited in GenBank under sequence read archive accession number PRJNA632691. Here is reported the draft genome sequence of *Brevibacillus borstelensis* GP-1 with 5.321 Mb of chromosomal content, with 51.50% GC content, without any extrachromosomal elements.

1. Introduction

Continuous demand for novel biomolecules, including enzymes, has directed researchers to explore more and more about extremophilic microorganisms. Hot springs are the most enchanting sources for thermophilic bacterial isolates with potential interests (Shahinyan et al., 2017; Panosyan et al., 2020). *Brevibacillus borstelensis* is recently been explored by many groups for its potential for the production of extracellular hydrolytic enzymes (Khalil, 2011; Norashirene et al., 2013). Aulitto et al. also reported *Brevibacillus borstelensis* SDM involved in lignocellulose transformation (Aulitto et al., 2020). *Brevibacillus* is a gram-positive, sporulating, non-capsulating, thermophilic bacterium, capable of producing many potential metabolites of industrial applications, including hydrolases, such as cellulase, amylase, and lipase (Norashirene et al., 2013) and have been reported to degrade polyethylene (Muhonja et al., 2018; Hadad et al., 2005). Genomic analyses of different strains of *Brevibacillus borstelensis* have been reported (Tripathy et al., 2016; Khalil et al., 2018). Lipase from *Brevibacillus borstelsnsis* strains has been reported to be immobilized on grapheme oxide and its activity and reusability studies are reported (Dutta and Saha, 2018; Nemetian et al., 2020). The current study focuses on the thermophilic lipase producing strain of *Brevibacillus borstelensis* and its whole genome sequencing for further work, which shall add to the few number of whole genome databases of *Brevibacillus borstelensis* available.

2. Materials and methods

2.1. Isolation of bacterium

A natural hot spring water sample was collected from Ganeshpuri (19.45 N, 72.79 E), Thane, Maharashtra, India, was serially diluted and spread on sterile Rhodamine B agar plates with the composition (g/L): Rhodamine B dye, 0.001; nutrient broth, 0.8; NaCl, 0.4; agar, 1 and olive oil, 3 (v/v) in distilled water, with pH 6.5. The plates were then incubated at 30 °C for 48 h. The colonies with fluorescent orange halos (under UV light (350 nm) exposure) were selected for further studies. The colonies were subcultured on the same medium. Pure isolated colonies were maintained on sterile nutrient agar slants with glycerol, under refrigeration.

Abbreviations: tRNA, transfer RNA; rRNA, ribosomal RNA; nc RNA, non-coding RNA; g/L, grams/litre; v/v, volume/volume; ORF, Open Reading Frame; NCBI, National Center for Biotechnology Information; PGAP, Prokaryotic Genome Annotation Pipeline; RAST, Rapid Annotation Server Test.

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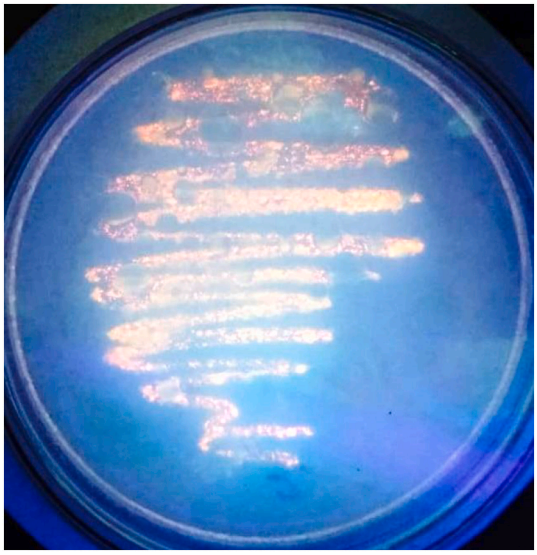

Fig. 1. Orange fluorescence of GP-1 on Rhodamine B agar plate upon exposure to UV light.

Table 1
Genome features of *Brevibacillus borstelensis* GP-1.

Genome features	GP-1
Genome size	5,321,525 bp
Total no. of scaffolds	141
G + C content	51.50%
Total no. of genes	5226
Total no. of protein-coding genes	4969
tRNA genes	112
rRNA genes	18
nc RNA	6
Pseudogenes	121
CRSIPR arrays	2

2.2. Genomic DNA isolation, sequencing & data assessment

DNA isolation was carried out using Xcelgen bacterial DNA isolation kit and quality was checked using agarose gel electrophoresis. Paired-end library preparation was done using Next Ultra DNA Library Prep kit for genome sequencing. The library was then analyzed in Bioanalyzer 2100 (Agilent Technologies) using a high sensitivity DNA chip. The genome library was then loaded onto an Illumina platform for cluster generation and sequencing.

De novo assembly of short reads was done using SoapDenovo v 2.04 and the assembly was optimized at Kmer-119. The scaffolds were further gap-filled using GapCloser v 1.12 software. ORF prediction and automatic annotation were performed using NCBI PGAP (http://www.ncbi.nlm.nih.gov/genome/annotation.prok). The genome was further analyzed using Rapid Server Annotation Test (RAST) and CG viewer software.

3. Results

Hot springs are a source of extremophilic microorganisms, potential sources of bacteria with hydrolytic enzyme production potential. A gram-positive, slight brown pigment-producing bacterial isolate, GP-1, showing lipolytic potential was isolated from Ganeshpuri hot water spring. The lipolytic potential was confirmed by the production of fluorescent orange halo around the colony on Rhodamine B agar, upon exposure to UV light (350 nm) (Fig. 1). Optimal growth of GP-1 was observed with Olive oil as a source of lipid, at pH 10 and temperature of 55 °C.

The analysis of raw reads obtained from the Illumina platform resulted in the single chromosome, with 141 scaffolds, with a genome size of 5.321 Mb and 51.50% GC content, without any extrachromosomal components.

Annotating the draft genome of *Brevibacillus borstelensis* GP-1 has revealed the presence of 5226 total genes, with 4969 protein-coding sequences (CDS) (Table 1). Out of these 4969 genes, 112 tRNAs, 18 rRNAs, and 6 nc RNA were identified. The assembly showed 121 pseudogenes and 2 CRISPR arrays. Subsystem coverage of these coding

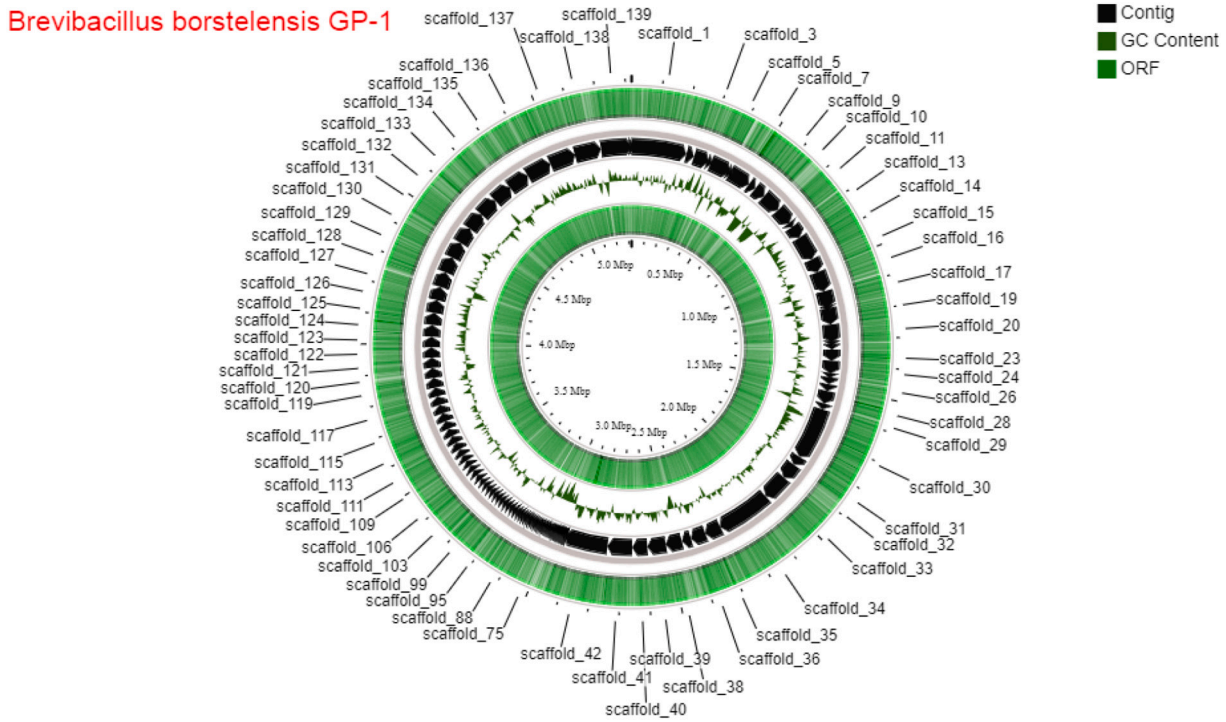

Fig. 2. Genome map of *Brevibacillus borstelensis* GP-1 using CG viewer software.

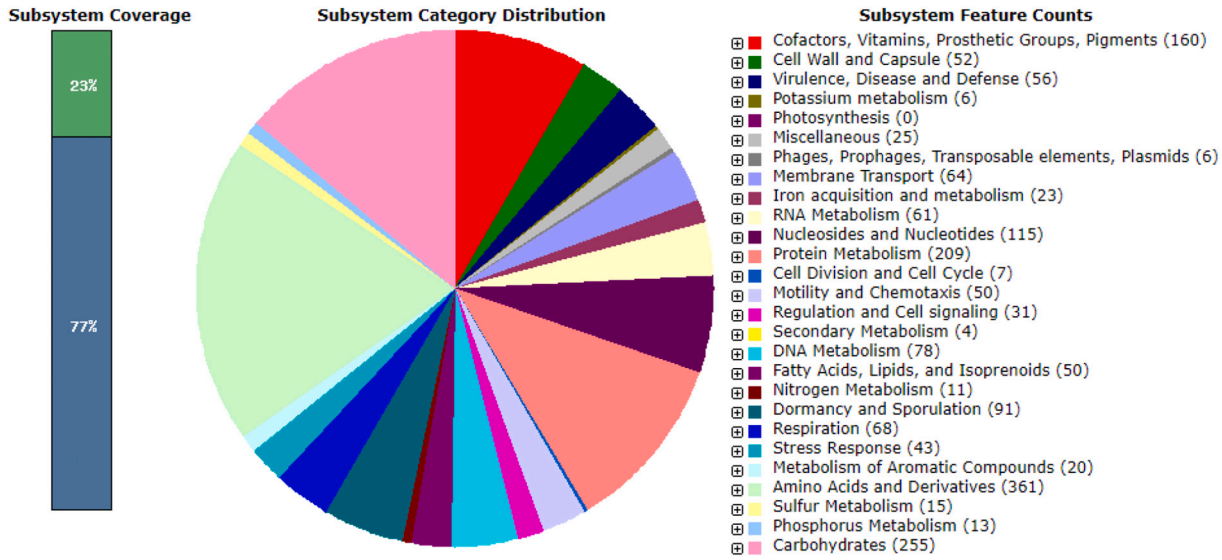

Fig. 3. RAST analysis of *Brevibacillus borstelensis* GP-1 strain.

sequences was about 23% involving at least 28 subsystem types. 50 of the CDS belonged to fatty acid and lipid metabolism (Fig. 3).

4. Discussion

Sequencing of strain GP-1 using Illumina platform generated a total sequence length of 5,321,525 and 5,321,409 without gaps. The closest neighbors as per the NCBI database are *Brevibacillus borstelensis* AK1 (92.6949% symmetrical identity) (Khalil et al., 2018) and *Brevibacillus borstelensis* 3096-7 (93.3507% symmetrical identity). *Brevibacillus borstelensis* GP-1 strain has a total of 5226 annotated genes. The CGView server is a comparative genomics tool for circular genomes, such as bacterial, that allows feature information to be visualized in the context of sequence analysis results and sequence similarity plots (Fig. 2).

The RAST analysis revealed 50 genes are involved in fatty acid, lipid and isoprenoid metabolism. RAST analysis showed 23% genes present in the subsystem, with a total of 1258 genes, including 1193 non-hypothetical and 65 hypothetical proteins; whereas 77% genes (4389 proteins) were not in the subsystem, including 2014 non-hypothetical and 2375 hypothetical proteins (Fig. 3). *Brevibacillus borstelensis* has been reported more recently for the production of various hydrolytic enzymes, including amylase, protease and lipase, and polyethylene degradation even (Shahinyan et al., 2017; Panosyan et al., 2020).

5. Conclusion

Extremophilic bacteria had been a potential source of valuable metabolites, particularly hydrolytic enzymes. The bacterial isolate *Brevibacillus borstelensis* GP-1, isolated from Ganeshpuri is a prospective candidate for extracellular lipase production. WGS analysis revealed 4969 protein-coding genes, and exploration of these shall hold the future potential of this bacterium at an industrial scale.

Nucleotide sequence accession numbers

The draft genome sequence for *Brevibacillus borstelensis* strain GP-1 has been deposited in DDBJ/EMBL/GenBank under the accession number JABGNB000000000, at scaffold level.

CRediT authorship contribution statement

Aparna J. Tailor: Conceptualization, Visualization, Investigation, Writing – original draft, Writing – review & editing, Methodology, Resources. **Rekha R. Gadhvi:** Supervision, Project administration. **Bhrugesh P. Joshi:** Formal analysis, Data curation, Validation.

Declaration of competing interest

The authors declare that there is no conflict of interest with reference to the work published in this paper.

References

Aulitto, M., Tom, L.M., Ceja-Navarro, J.A., Simmons, B.A., Singer, S.W., 2020. Whole genome sequence of *Brevibacillus borstelensis* SDM, isolated from a sorghum-adapted microbial community. Microb. Resour. Announc. 9, e01046-20 https://doi.org/10.1128/MRA.01046-20.

Dutta, N., Saha, M.K., 2018. Immobilization of a mesophilic lipase on graphene oxide: stability, activity and reusability insights. In: Methods in Enzymology, 609, pp. 247–272. https://doi.org/10.1016/bs.mie.2018.05.009.

Hadad, D., Geresh, S., Sivan, A., 2005. Biodegradation of polyethylene by thermophilic bacterium *Brevibacillus borstelensis*. J. Appl. Microbiol. 98, 1093–1100. https://doi.org/10.1111/j.1365-2672.2005.02553.x.

Khalil, A., 2011. Isolation and characterization of three thermophilic bacterial strains (lipase, cellulase and amylase producers) from hot springs of Saudi Arabia. Afr. J. Biotechnol. 10 (44), 8834–8839. https://doi.org/10.5897/AJB10.1907.

Khalil, A.B., Sivakumar, N., Arslan, M., Saleem, H., Qarawi, S., 2018. Insights into *Brevibacillus borstelensis* AK1 through whole- genome sequencing: a thermophilic bacterium isolated from a hot spring in Saudi Arabia. Biomed. Res. Int. https://doi.org/10.1155/2018/5862437.

Muhonja, C.N., Makonde, H., Magoma, G., Imbuga, M., 2018. Biodegradability of polyethylene by bacteria and fungi from Dandora dumpsite, Nairobi, Kenya. PLoS ONE 13 (7), e0198446. https://doi.org/10.1371/journal.pone.0198446.

Nemetian, T., Shakeri, A., Salehi, Z., Saboury, A.A., 2020. Lipase immobilized on functionalized supermagnetic few layer grapheme oxide as an efficient nanobiocatalyst for biodiesel production from *Chlorella vulgaris* bio-oil. In: Biotechnology for Biofuels, 13, p. 57. https://doi.org/10.1186/s13068-020-01688-x.

Norashirene, M.J., Umi Sarah, M.H., Khairiyah, S., Nurdiana, S., 2013. Biochemical characterization and 16S rDNA sequencing of lipolytic thermophiles from Selyang hot spring, Malaysia. IERI Procedia 5, 258–264. https://doi.org/10.1016/j.ieri.2013.11.101.

Panosyan, H., Margaryan, A., Blrkeland, N.K., 2020. Geothermal springs in Armenia and Nagorno-Karakabh: potential sources of hydrolase producing thermophilic bacilli. Extremophiles. https://doi.org/10.1007/s00792-020–01173-1.

Shahinyan, G., Margaryan, A., Panosyan, H., Trchounian, A., 2017. Identification and sequence analyses of novel lipase encoding novel thermophilic bacilli isolated from Armenian geothermal springs. BMC Microbiol. 17, 103. https://doi.org/10.1186/s12866-017-1016-4.

Tripathy, S., Padhi, S.K., Sen, R., Maji, U., Samanta, M., Mohanty, S., Maiti, N.K., 2016. Draft genome sequence of *Brevibacillus borstelensis* cifa_chp40, a thermophilic strain having biotechnological importance. J. Genom. 4, 4–6. https://doi.org/10.7150/jgen.14036.