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ORIGINAL RESEARCH ARTICLE

Genome Sequencing Revealed the Biotechnological Potential of an Obligate Thermophile *Geobacillus thermoleovorans* Strain RL Isolated from Hot Water Spring

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Abstract In the present study, we report the draft genome sequence of an obligate thermophile *Geobacillus thermoleovorans* strain RL isolated from Manikaran hot water spring located atop the Himalayan ranges, India. Strain RL grew optimally at 70 °C but not below 45 °C. The draft genome (3.39 Mb) obtained by Illumina sequencing contains 138 contigs with an average G + C content of 52.30%. RAST annotation showed that amino acid metabolism pathways were most dominant followed by carbohydrate metabolism. Genome-wide analysis using NCBI's Prokaryotic Genome Annotation Pipeline revealed that strain RL encodes for a cocktail of industrially important hydrolytic enzymes glycoside hydrolase, α-and β-glucosidase, xylanase, amylase, neopullulanase, pullulanase and lipases required for white biotechnology. In addition, the presence of genes encoding green biocatalyst multicopper polyphenol oxidase (laccase) and an anticancer enzyme L-glutaminase reflects the significance of strain RL in gray and red biotechnology, respectively. Strain RL is a thermophilic multi-enzyme encoding bacterium which could be the source for the recombinant production of biotechnologically significant enzymes. In, addition whole cells of strain RL may be used in bioremediation studies.

Keywords Thermophile · *Geobacillus thermoleovorans* · Draft genome · Hydrolytic enzymes · Bioremediation · Biotechnology

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Introduction

The thermophilic microorganisms possess several properties which have made them suitable for commercial applications [1]. The natural habitat for thermophiles ranges from deep volcanoes to hydrothermal systems and hot water springs. The human-created environments including compost, different industrial processes and water heaters are some other important habitat of thermophiles [1, 2]. As a consequence of their unique physiological adaptations, thermophilic microorganisms have enzymes which withstand high temperature. Thermostable enzymes are industrially important due to their stability under different processing conditions. The great stability of thermozymes under high temperatures, acidic and alkaline pH, solvents and detergents has raised the demand for bio-prospection of enzymes from thermophilic microorganisms [3, 4].

In recent years, thermophilic *Geobacillus* species have emerged as sources of various thermostable enzymes [5–11]. *Geobacillus stearothermophilus* has been extensively studied for xylanase production [6, 12, 13]. In addition, *Geobacillus* sp. HTA426 has been used for the production of thermophilic cellulase [14]. Moreover, *Geobacillus* species are known for the production of bacteriocins, exopolysaccharides, and biofuel, and also

involved in bioremediation, adding new applications to the continually evolving group [5]. Our group has studied the culturable and unculturable bacterial and viral diversity of Manikaran hot springs located atop the Himalayan ranges in India [15–22]. The major objective of the present study was isolation and characterization of thermophilic *Geobacillus* species which could be used as a source for the production of cocktails of thermostable enzymes required in different sector of biotechnology.

Materials and Methods

Isolation, Identification and Genome Sequencing

In order to isolate obligate thermophiles, water samples were collected from Manikaran hot water spring (31°20′25″ to 32°25′0″ north latitude and 76°56′30″ to 77°52′20″ east longitude) located atop the Himalayan ranges, Himachal Pradesh, India. Serially diluted water sample was spread on Thermus agar (0.8% polypeptone, 0.4% yeast extract, 0.2% NaCl and 0.1% glucose, pH 7.2) plates. The plates were incubated at 70 °C for 48 h. To replace water loss during incubation a flask containing 500 mL distilled water was kept inside the incubator [20]. The isolate optimally growing at 70 °C was identified based on 16S rRNA gene sequence [23] and average nucleotide identity (ANI) [24, 25]. The genomic DNA was extracted using the Qiagen genomic DNA extraction kit (Cat No. 51304) as per the manufacturer's protocol. After the quality check, library preparation was carried out at Bionivid Technology Pvt. Ltd (Bangalore) India. The paired-end sequencing was performed using the Illumina HiSeq 2500 sequencing platform.

Genome Assembly and Annotation

The high-quality reads obtained after trimming of adaptor sequences were assembled at different k-mers (k = 31 to k = 99) using AbySS 2.1.5 [26]. The optimized assembly was validated by mapping raw reads against assembled contigs using Burrows–Wheeler aligner [27]. The final assembly was annotated using Rapid Annotations using Subsystems Technology (RAST) and NCBI's Prokaryotic Genome Annotation Pipeline (PGAP) version 4.0 [28, 29].

Accession Number

The draft genome sequence of *Geobacillus thermoleovorans* strain RL is available in GenBank/DDBJ/EMBL under accession number SZVQ00000000.

Results and Discussion

Description of the Draft Genome

Based on 16S rRNA gene sequence, the isolate optimally growing at 70 °C showed 100% identity with *Geobacillus thermoleovorans* KCTC3570 (Accession: CP014335.1). Strain described in this study was designated as *Geobacillus thermoleovorans* strain RL which showed 98.59% ANI value with strain KCTC3570. Genome sequencing resulted in a total of 37,642,234 filtered paired-end raw reads. The raw reads, assembled using AbySS 2.1.5 generated a consensus assembly at k = 95 (N50 = 58,563 bp; L50 = 17). The draft genome consists of 138 contigs with a total size of 3,392,277 bp. The average chromosome G + C content was 52.30%. Using PGAP a total of 3575 genes were predicted, including 3249 protein-coding genes (PCGs), 16 rRNA subunits (8 genes for 5S and 4 genes each for 16S, and 23S subunits), 79 tRNAs, 5 noncoding RNAs, and 226 pseudogenes. Genome annotation revealed the presence of genes encoding different biotechnologically important enzymes that are described in the next section. Enzymes derived from thermophilic microorganisms are highly stable than similar enzymes obtained from mesophilic microorganisms. At higher operational temperature thermostable enzyme catalysed reactions have a higher reaction rate as compared to mesophilic counterparts [30].

Biotechnological Potential of *G. thermoleovorans* Strain RL

Bio-conversion of lignocellulosic material to biofuels is a feasible approach which efficiently reduces environment polluting greenhouse gases [31]. Strain RL has different enzymes required for the saccharification of lignocellulosic biomass that cannot be easily degraded due to the recalcitrant nature of their structural components viz cellulose and hemicelluloses. Enzymatic cleavage of cellulose and hemicelluloses yields fermentable sugars required in different industrial processes [32]. In general, *Geobacillus* species does not exhibit factual cellulolytic activity (hydrolysis of crystalline cellulose) but glycoside hydrolases that degrade non-crystalline polymers into short oligomers which are easily transported inside the cell are secreted. These short chain oligomers are further hydrolysed to monomers by glycosidases and non-secreted glycoside hydrolase present inside the cell. In this study, strain RL harbours multiple glycoside hydrolases (FDK15_12275, FDK15_16585; FDK15_00760; FDK15_00825; FDK15_01245; FDK15_01335) and hydrolases (FDK15_14865; FDK15_01440; FDK15_01480; FDK15_02225; FDK15_02465) which catalyses the hydrolysis of glycosidic linkages present in

complex polymers. Moreover, the presence of xylanolytic enzyme system reflects the hemicellulose degrading activity of strain RL. Hemicelluloses are heteropolysaccharide primarily composed of xylan, galactan, mannan, and arabinan. Xylan is the most common hemicellulosic polysaccharide composed of D-xylose subunits linked with β-1,4-glycosidic linkages [33]. Due to the heterogeneous and complex chemical nature of xylan, its hydrolysis requires the cooperative action of different hydrolytic enzymes [33]. The xylan degradation potential of strain RL was reflected by an arsenal of xylanolytic enzymes including β-1,4-endoxylanase (FDK15_01205; FDK15_02325) β-xylosidase (FDK15_00720), α-L-arabinofuranosidase (FDK15_00785; FDK15_00795) and acetyl xylan esterase (FDK15_08785) which act synergistically for the conversion of xylan into its constituent sugars. Besides strain RL possessed multiple copies of genes encoding α-amylase (FDK15-05125) suggesting starch hydrolysing potential. Further, gene encoding pullulanase (FDK15_14705) reflected the wide ability of strain RL towards hydrolysis of α-1,6 glycosidic bonds present in starch, amylopectin and pullulan [34]. Multiple copies of neopullulanase (FDK15_01830; FDK15_01850) involved in the cleavage of both 1–4 and 1–6 linkages reflected the cyclodextrin hydrolysing abilities of strain RL [35–37]. In addition, strain RL possess lipases (FDK15_01215; FDK15_09115; FDK15_10720; FDK15_13240; FDK15_14380) that catalyses the transesterification reaction resulting in the production of biodiesel (alkyl esters) [38].

In addition, strain RL harbours genes encoding multicopper polyphenol oxidase (Laccase) (FDK15_03000) and multicopper oxidase (FDK15_04830) which have several applications in the area of grey biotechnology viz, lignification, delignification, detoxification of recalcitrant pollutants and decolorization of wastewater [2, 39–43]. The diversity of laccases in bacteria isolated from hot sulphur springs located in Leh, India has been reported and bioprospection of thermostable laccases is a continuous process [2]. Interestingly, the presence of genes encoding L-glutaminase (FDK15_12655) indicates the utility of strain RL in the pharma industry. L-glutaminase used in the treatment of lymphocytic leukemia hydrolyzes L-glutamine to L-glutamate and ammonia [44, 45]. The anticancer effect is due to depletion of L-glutamine in cancerous cells which cannot synthesize L-glutamine but utilizes it more efficiently than normal cells [44, 46, 47].

Conclusion

We report the draft genome sequence of an obligate thermophilic bacterium *Geobacillus thermoleovorans* strain RL. Strain RL genome encodes for multiple enzymes of biotechnological significance which include glycoside hydrolase, α-and β-glucosidase, xylanase, amylase, neopullulanase, pullulanase, and lipases. In addition, strain RL encodes for green biocatalyst multicopper polyphenol oxidase (laccase) and an anticancer enzyme L-glutaminase. This study reflects the implications of strain RL in white, grey and red biotechnology.

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Compliance with Ethical Standards

Conflict of interest All authors declare no conflict of interest.

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