Comparative Genomic Analysis of Arctic Permafrost Bacterium *Nesterenkonia* sp. PF2B19 to Gain Insights into Its Cold Adaptation Tactic and Diverse Biotechnological Potential

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Abstract: *Nesterenkonia* sp. PF2B19, a psychrophile was isolated from 44,800-year-old permafrost soil. This is the first report on comparative genomics of *Nesterenkonia* sp. isolated from Arctic. Genome of PF2B19 exhibited the presence of a vast array of genetic determinants involved in cold adaptation i.e., response to cold-associated general, osmotic, and oxidative stress. These genomic attributes proved to be valuable in unraveling the adaptive tactics employed by PF2B19 for survival in the cold permafrost soils of the Arctic. Genomic analysis of PF2B19 has given some valuable insight into the biotechnological potential of this strain, particularly as a source of cold-active enzymes, as a bioremediating agent and as plant growth-promoting bacteria.

Keywords: *Nesterenkonia* sp.; permafrost; comparative genomics; cold adaptation

1. Introduction

Permafrost defines soil, rock or sediment that is frozen for more than two consecutive years [1], covering >25% of the land surface in the northern hemisphere [2]. Harsh conditions prevail in such soils like nutrient limitation, extreme aridity and pH, low temperature, high ultraviolet irradiation, etc. [3,4]. In spite of such extreme conditions, reports suggest the presence of metabolically-active microbial life in the permafrost soil of Svalbard [5,6]. Permafrost soils are considered as chronological collections of past and present microbes [7]. These soils are characterized as extreme environments which can severely impair the cellular function by negatively affecting the cell integrity, membrane fluidity, enzyme kinetics and other interactions [8]. Therefore, for an organism to survive and grow in such extreme niches, it should harbor genes encoding enzymes involved in regulation of metabolically-active microbial life in the permafrost soil of Svalbard [5,6]. Permafrost soils are considered as chronological collections of past and present microbes [7]. These soils are characterized as extreme environments which can severely impair the cellular function by negatively affecting the cell integrity, membrane fluidity, enzyme kinetics and other interactions [8]. Therefore, for an organism to survive and grow in such extreme niches, it should harbor genes encoding enzymes involved in regulation of DNA replication, transcription, translation and membrane fluidity at low temperatures and other stress combative mechanisms. The microorganisms harboring such harsh microenvironments have evolved certain adaptive features to combat various cold environment-related stresses such as cold stress, oxidative stress, osmotic stress, low nutrient availability, etc. [9,10].

In the last few decades, there has been a growing interest in permafrost as it is known to harbor potentially novel and biotechnologically important microorganisms [11]. Psychrophiles are the most probable sources of cold-active enzymes [12]. These cold-active enzymes have high catalytic efficiency and stability at low and moderate temperatures [13]. Cold-active enzymes have huge market potential as compared to mesophilic and thermophilic enzymes as they shorten process time and cut down energy costs. These enzymes
find wide applications in biotechnological and industrial usage, especially in detergents, cosmetics, textiles, etc.

Although permafrosts are known to cover 27% of the Earth [14], there are very few reports on bacterial community composition of permafrost soil from Svalbard (78° N) [15,16]. Additionally, genomes sequenced from cold environments are relatively few [17]. The molecular strategy employed by bacteria for cold-adaptation in such harsh environments remains poorly understood. Genus *Nesterenkonia* belongs to the family *Micrococcaceae*, within the phylum *Actinobacteria* [18]. *Nesterenkonia* sp. is coccoid, aerobic and non-spore forming bacteria [18,19]. At present, only nine genomes of *Nesterenkonia*, sp. are available publicly. Reports suggest that some of the *Nesterenkonia* strains are associated with extreme environments underlining their importance as sources of industrially important cold active enzymes [20].

In this study, a psychrophilic bacterium, *Nesterenkonia* sp. strain PF2B19 was isolated from permafrost soil. Here, we attempted, by means of genome sequencing of this strain, to unravel the molecular machineries associated with cold adaptation and to identify industrially important cold-active enzymes.

2. Materials and Methods

2.1. Sampling Site, Bacterial Strain and Growth Conditions

*Nesterenkonia* sp. PF2B19 (PF2-B6) was isolated from permafrost soil gathered from Svalbard, Arctic (78°55.165′ N, 11°52.660′ E) on 20 August 2007. This strain was cultured routinely at 15 °C on Zobell Marine Agar. The pure culture of *Nesterenkonia* sp. PF2B19 has been deposited with accession number MCC 3408 at Microbial Culture Collection (MCC), India.

2.2. Genomic DNA Preparation and Genome Sequencing

Genomic DNA from the strain PF2B19 was isolated using GenElute™ Bacterial Genomic DNA Isolation kit (Sigma, St. Louis, MO, USA). The PF2B19 genome was sequenced on the Ion Torrent PGM platform (Life Technologies, Carlsbad, CA, USA) using the 316™ chip and 200-bp chemistry. The obtained sequence was then de novo assembled using SPAdes assembler version 3.9.1 [21].

2.3. Comparative Genomics

Digital DNA-DNA Hybridization was executed as described by Auch et al. (2010) [22] using online tool http://ggdc.dsmz.de (accessed on 3 March 2021) with PF2B19 as query genome and *Nesterenkonia* JCM 19054, *Nesterenkonia alba* DSM 19423(T), *Nesterenkonia massiliensis* strain NP1, *Nesterenkonia* sp. AN1, *Nesterenkonia* sp. F and *Nesterenkonia jeotgali* CD08_7 as reference genomes. Genome sequence of PF2B19 further compared with the genomes of above mentioned strains in RAST tool to determine distinctive genomic determinants, i.e., gene unique in PF2B19 to prove its novelty. A circular map representing the general genome comparisons of strain PF2B19 with its close phylogenetic affiliates (*Nesterenkonia* JCM 19054, *Nesterenkonia alba* DSM 19423(T) and *Nesterenkonia* sp. AN1) was generated using the BRIG program. BRIG uses BLAST for genome comparisons and CGView for image generation. The circular image is generated wherein the reference genome is placed at the center and other query genomes as a set of concentric rings colored displaying similarity. The genomes of reference *Nesterenkonia* strains NP1, F, AN1, JCM 19054, DSM 19423 and CD08_7 were obtained from the NCBI database.

2.4. Functional Annotation

Functional annotation of PF2B19 genome was carried out by Rapid Annotation using Subsystem Technology (RAST) [23]. PF2B19 genome was mined for the presence of genes having role in cold adaptation and biotechnological potential in RAST annotation tool. Pathway elucidation was executed using Kyoto Encyclopedia of Genes and Genomes (KEGG)
PF2B19, a Gram positive, strictly aerobic coccoid, was identified as the affiliate of the psychrophilic genus *Nesterenkonia* based on 16S rRNA gene sequencing, displaying maximum 16S rRNA sequence (1312 nucleotides) homology of 99% with closest phylogenetic neighbors *Nesterenkonia aethiopica* DSM 17733(T), *Nesterenkonia xinjiangensis* strain YIM70097, *Nesterenkonia* sp. YIM70097 and *Nesterenkonia suensis* Sua-BAC020(T). PF2B19 shared 98% homology with *Nesterenkonia massiliensis* strain NP1. 16S rRNA gene sequences of PF2B19 were aligned with those of the publicly available *Nesterenkonia* 16S rRNA sequences using the Mega version 6.0 [25] Phylogenetic tree showing the taxonomic relationship of strain PF2B19 with other *Nesterenkonia* strains was constructed by employing the neighbor-joining algorithm (Figure 1).

![Phylogenetic tree](image)

**Figure 1.** Phylogenetic tree displaying the taxonomic relationship between PF2B19 and other related members of the genus *Nesterenkonia*. (*Halostagnicola larsenii* JCM 13463 was used as an outgroup).

However, the whole genomes of *Nesterenkonia aethiopica* DSM 17733(T), *Nesterenkonia xinjiangensis* strain YIM70097, *Nesterenkonia* sp. YIM70097 and *Nesterenkonia suensis* Sua-BAC020(T) are not available in the NCBI database, so *Nesterenkonia* JCM 19054, *Nesterenkonia alba* DSM 19423(T), *Nesterenkonia massiliensis* strain NP1, *Nesterenkonia* sp. AN1, *Nesterenkonia* sp. F and *Nesterenkonia jeotgali* CD08_7 were selected for Digital DNA–DNA hybridization. Digital DNA–DNA hybridization revealed homology of only 27.50%, 23.10%, 24.90%, 24.50%, 26.30% and 24.70% between PF2B19 and *Nesterenkonia* JCM 19054, *Nesterenkonia alba* DSM 19423(T), *Nesterenkonia massiliensis* strain NP1, *Nesterenkonia* sp. AN1, *Nesterenkonia* sp. F and *Nesterenkonia jeotgali* CD08_7 respectively, outlining the difference between the species and also illustrating the novelty of strain PF2B19. Based
on this information, PF2B19 can be considered as a putative novel species of the genus *Nesterenkonia*.

### 3.2. General Genome Features of Permafrost Bacterium *Nesterenkonia* sp. PF2B19

Sequencing of the library generated 3,698,032 bp reads, which were de novo assembled using SPAdes assembler version 3.9.1 into 135 contigs, yielding a 3.6 Mb genome with 69.5% G+C content. These results were in congruence with publicly available draft genomes of three strains of *Nesterenkonia* possessing sizes in the range of 2.59 to 3.01 Mb and G+C contents of 62.2 to 71.5%. Functional annotation of PF2B19 genome by RAST revealed a total of 3763 proteins were predicted, including 3708 coding sequences and 55 total RNAs. Differentiating genome features of query genome PF2B19 along with five reference genomes are illustrated in Table 1.

| Attributes                     | Strains of the Genus *Nesterenkonia* * | PF2B19 | CD08_7 | AN1 | F | JCM 19054 | NP1 | DSM 19423 |
|-------------------------------|----------------------------------------|--------|--------|-----|---|-----------|-----|-----------|
| Accession no.                 |                                        | MDSS000000000 | LQBM000000000 | JEMO000000000 | AFRW000000000 | BAXI000000000 | CBLL000000000 | ATXP000000000 |
| Isolation source              |                                        | Permafrost soil Svalbard, Arctic | Duodenal mucosa of CD patient | Salt Lake, Iran | Antarctic soil | Sea snail *Nassarius glans* | Feces of AIDS patient | Black liquor treatment system of a cotton pulp mill |
| Growth temp                   |                                        | 15 °C | 37 °C | 21 °C | 32 °C | 28 °C | 37 °C | 42 °C |
| Size                          |                                        | 3.6 Mb | 2.9 Mb | 3.0 Mb | 2.8 Mb | 2.5 Mb | 2.6 Mb | 2.5 Mb |
| Contigs                       |                                        | 135 | 8 | 42 | 138 | 1086 | 175 | 36 |
| G+C (%)                       |                                        | 69.5 | 67.6 | 67.4 | 71.5 | 67.1 | 62.9 | 63.7 |
| No. of RNAs                   |                                        | 55 | 52 | 52 | 50 | 48 | 49 | 51 |
| No. of subsystem              |                                        | 394 | 379 | 374 | 347 | 292 | 355 | 343 |
| Coding sequences              |                                        | 3708 | 2531 | 2846 | 2480 | 3901 | 2435 | 2295 |

* *Nesterenkonia* sp. PF2B19; *Nesterenkonia* jeotgali CD08_7; *Nesterenkonia* sp. AN1; *Nesterenkonia* sp. F; *Nesterenkonia* sp. JCM 19054; *Nesterenkonia* massiliensis NP1; *Nesterenkonia* alba DSM 19423.

### 3.3. General Genome Comparisons of PF2B19 with Its Closest Phylogenetic Affiliates

PF2B19 genome was compared with the available *Nesterenkonia* genomes, by running BLASTn in BRIG software [26]. The circular map (Figure 2) represents the BLASTn results of each query genome (*Nesterenkonia* JCM 19054, *Nesterenkonia* alba DSM 19423(T) and *Nesterenkonia* sp. AN1) against the reference PF2B19. As evident from the BRIG image, gaps were more pronounced in the query genomes, emphasizing the difference between PF2B19 and the other *Nesterenkonia* genomes.
3.4. Comparative Genomics Identifies Unique Genes/Proteins in Nesterenkonia sp. PF2B19

Genome annotation performed using RAST tool identified *Renibacterium salmoninarum* ATCC 33209 (Genome id: 288705.3, Score: 512) as the closest phylogenetic neighbor of PF2B19. On comparative analysis with ATCC 33209, 378 unique genes associated with a subsystem in PF2B19 were detected in PF2B19. These genes were scored as distinctive genomic determinants that differentiated PF2B19 from its phylogenetic associates.

PF2B19 genome was also compared with other *Nesterenkonia* genomes in RAST. Unique genes were detected in PF2B19 as compared to other *Nesterenkonia* sp., further highlighting the novelty of PF2B19 (Table 2).

Table 2. Unique genes detected in PF2B19 genome on comparison to available *Nesterenkonia* genomes.

| Genome Used for Comparison | No. of Unique Genes Detected in PF2B19 on Comparison |
|----------------------------|------------------------------------------------------|
| *Nesterenkonia alba* DSM19423 | 323 |
| 1. Betaine aldehyde dehydrogenase (EC 1.2.1.8) | |
| 2. Glycine betaine ABC transport system permease protein | |
| 3. Glycine betaine transporter OpuD | |
| 4. Choline dehydrogenase (EC 1.1.99.1) | |
| 5. Sarcosine oxidase alpha, beta and gamma subunit (EC 1.5.3.1) | |

These genes help counteract cold-induced osmotic stress.
### Table 2. Unique genes detected in PF2B19 genome on comparison to available Nesterenkonia genomes.

| Genome Used for Comparison | No. of Unique Genes Detected in PF2B19 on Comparison | Type of Distinct Genes Detected in Relation to Psychrophilic Lifestyle of PF2B19 | Role |
|-----------------------------|-------------------------------------------------------|---------------------------------------------------------------------------------|------|
| *Nesterenkonia alba* DSM 19423(T) | 323 | 1. Betaine aldehyde dehydrogenase (EC 1.2.1.8)  
2. Choline dehydrogenase (EC 1.1.99.1)  
3. Sarcosine oxidase alpha, beta and gamma subunit (EC 1.5.3.1)  
4. Ectoine hydroxylase (EC 1.17.1.7)  
5. Glutathione synthetase (EC 6.3.2.3)  
6. Hydroxy acyl glutathione hydrolase (EC 3.1.2.6)  
7. Lactoylglutathione lyase (EC 4.4.1.5)  
8. Redox-sensitive transcriptional activator SoxR  
9. Transcriptional regulator, Crp/Fnr family  | Counteract against cold-induced osmotic stress  
Counteract against cold-induced oxidative stress  
Modulate membrane fluidity at low temperatures  |  

| *Nesterenkonia massilesis* NP1 | 310 | 1. Starvation sensing protein RspA  | Carbon Starvation  
Counteract against cold-induced osmotic stress  
Counteract against cold-induced oxidative stress  |
Table 2. Cont.

| Genome Used for Comparison | No. of Unique Genes Detected in PF2B19 on Comparison | Type of Distinct Genes Detected in Relation to Psychrophilic Lifestyle of PF2B19 | Role |
|----------------------------|-----------------------------------------------------|--------------------------------------------------------------------------------|------|
| Nesterenkonia sp. F        | 215                                                 | 1. Outer membrane protein A precursor                                           | Counteract against cold-induced osmotic stress |
|                            |                                                     | 1. Glutathione synthetase (EC 6.3.2.3)                                          |                                               |
|                            |                                                     | 2. Alkyl hydroperoxide reductase subunit C-like protein                         |                                               |
|                            |                                                     | 3. Redox-sensitive transcriptional activator SoxR                               |                                               |
|                            |                                                     | 4. Superoxide dismutase [Cu-Zn] precursor (EC 1.15.1.1)                         |                                               |
|                            |                                                     | 5. Transcriptional regulator, Crp/Fnr family                                    |                                               |
|                            |                                                     | Counteract against cold-induced oxidative stress                               |                                               |
|                            |                                                     | 1. Starvation sensing protein RspA                                               | Carbon starvation                             |
|                            |                                                     | 1. Geranylgeranyl diphosphate synthase (EC 2.5.1.29)                            | Modulate membrane fluidity at low temperatures |
| Nesterenkonia jeotgali CD08_7 | 218                                                 | 1. Sarcoisne oxidase alpha, beta and gamma subunit (EC 1.5.3.1)                | Counteract against cold-induced osmotic stress |
|                            |                                                     | 2. Outer membrane protein A precursor                                           |                                               |
|                            |                                                     | 1. Redox-sensitive transcriptional activator SoxR                               | Counteract against cold-induced oxidative stress |
|                            |                                                     | 1. Starvation sensing protein RspA                                               | Carbon starvation                             |
| Nesterenkonia sp. AN1      | 202                                                 | 1. Sarcoisne oxidase alpha, beta and gamma subunit (EC 1.5.3.1)                | Counteract against cold-induced osmotic stress |
|                            |                                                     | 2. Ectoine hydroxylase (EC 1.17.-.-)                                            |                                               |
|                            |                                                     | 3. Outer membrane protein A precursor                                           |                                               |
|                            |                                                     | 1. Redox-sensitive transcriptional activator SoxR                               | Counteract against cold-induced oxidative stress |
|                            |                                                     | 1. Starvation sensing protein RspA                                               | Carbon starvation                             |

| Genome Used for Comparison | No. of Unique Genes Detected in PF2B19 on Comparison | Type of Distinct Genes Detected in Relation to Psychrophilic Lifestyle of PF2B19 | Role |
|----------------------------|-----------------------------------------------|---------------------------------------------------------------------------------|------|
| *Nesterenkonia* sp. JCM 19054 | 345 | 1. Cold shock protein CspA | Cold shock response |
|                            |                                               | 1. Glycine betaine transporter OpuD                                            | Counteract against cold-induced osmotic stress |
|                            |                                               | 2. Sarcosine oxidase alpha, beta and gamma subunit (EC 1.5.3.1)               | |
|                            |                                               | 3. Ectoine hydroxylase (EC 1.17.-.-)                                           | |
|                            |                                               | 4. Outer membrane protein A precursor                                           | |
|                            |                                               | 1. Lactoylglutathione lyase (EC 4.4.1.5)                                       | Counteract against cold-induced oxidative stress |
|                            |                                               | 2. Redox-sensitive transcriptional activator SoxR                             | |
|                            |                                               | 3. Transcriptional regulator, FUR family                                       | |
|                            |                                               | 4. Transcriptional regulator, Crp/Fnr family                                  | |
|                            |                                               | 1. Starvation sensing protein RspA                                             | Carbon starvation |
3.5. Identification of Virulence Determinants

No virulent genes were detected in the genome of PF2B19 as revealed by Virulence Finder. Thus, PF2B19 was non-pathogenic.

3.6. Genes Involved in Resistance to Antibiotics

Antibiotic-resistance genes are potentially transferable genes in specific niches such as intestinal microflora where microbial inhabitants are often exposed to an exhaustive use of antibiotics. Yet, current studies have revealed the presence of antibiotic-resistant genes and/or antibiotic-resistance bacteria in the geographically isolated natural niches which are not exploited by anthropogenic factors [27–30]. We screened the genome of PF2B19, which was isolated from Arctic for presence of antibiotic-resistance genes. Interestingly, genes conferring resistance to fluoroquinolones and Beta lactam group of antibiotics were detected in PF2B19 genome. Presence of mutant genes: DNA gyrase subunit B (gyrB) (EC 5.99.1.3) and DNA gyrase subunit A (gyrA) (EC 5.99.1.3) and Topoisomerase IV subunit A (EC 5.99.1.-) and Topoisomerase IV subunit B (EC 5.99.1.-) were thought to be involved in conferring resistance against fluoroquinolone, while mutant Beta-lactamase class C and other penicillin-binding proteins were responsible for resistance towards Beta lactam group of antibiotics. Svalbard, Arctic, is not yet exploited by anthropogenic activities and the presence of antibiotic-resistance genes in the bacteria isolated from such a pristine environment was quiet surprising. Most probable modes of transmission would be through airborne bacteria and migratory birds.

Arctic is characterized by harsh cold conditions. The stresses encountered by bacteria in permafrost soil include limited nutrients, desiccation, oxidative stress, osmotic stress and persistent low temperatures [31,32]. A repertoire of adaptive genes associated with diverse stresses present in cold milieus have been reported in the literature [33–37]. PF2B19 genome was mined for the adaptive genes that may be associated with survival of PF2B19 in the permafrost soils of Svalbard. Analysis of Nesterenkonia sp. PF2B19 genome revealed a total of 128 putative stress response genes, including 16 genes linked to cold stress response, 16 genes for DNA repair, 12 genes for modulation of membrane fluidity, 39 genes for oxidative stress response, 37 genes for osmotic stress response and 4 in response to general stress (Table 3).

| Gene Name | Gene Products                        | Function         |
|-----------|-------------------------------------|------------------|
| cshA      | Putative ATP-dependent RNA helicase |                  |
| cspC      | Cold shock protein C                |                  |
| cspA      | Cold shock protein A                |                  |
| infB      | Translation initiation factor 1     |                  |
| deaD      | DEAD-box ATP-dependent RNA helicase |                  |
| Pnp       | Polyribonucleotide nucleotidyl transferase |              |
| infB      | Translation initiation factor 2     |                  |
| rfbA      | Ribosome-binding factor A           |                  |
| nusA      | Transcription termination protein   |                  |
| dnaJ      | Chaperone protein                   |                  |
| dnaK      | Chaperone protein                   |                  |
| grpE      | Heat shock protein                  |                  |
| hrpA      | ATP-dependent helicase              |                  |
| ygcA      | RNA methyltransferase, TrmA family  |                  |
| cstA      | Carbon starvation protein A         |                  |
| hrpA      | ATP-dependent helicase              |                  |

Table 3. Cold-induced stress associated genes in Nesterenkonia sp. PF2B19 genome.
| Gene Name | Gene Products | Function |
|-----------|---------------|----------|
| recA      | Recombinase   |          |
| recN      | DNA repair protein |          |
| recR      | Recombination protein |          |
| uvrA      | Excinuclease ABC subunit A paralog of unknown function | DNA repair |
| xthA      | Exodeoxyribonuclease III |          |
| mutM      | Formamidopyrimidine-DNA glycosylase |          |
| mutY      | A/G-specific adenine glycosylase |          |
| recA      | RecA protein |          |
| recX      | Regulatory protein |          |
| uvrC      | Excinuclease ABC subunit C |          |
| uvrB      | Excinuclease ABC subunit B |          |
| uvrA      | Excinuclease ABC subunit A |          |
| ruvA      | Holliday junction DNA helicase |          |
| ruvB      | Holliday junction DNA helicase |          |
| ruvC      | Crossover junction endodeoxyribonuclease |          |
| recO      | DNA recombination and repair protein |          |
| Pdg       | Endonuclease III |          |
| –         | Phytoene dehydrogenase and related proteins | Membrane fluidity |
| –         | Fatty acid desaturase |          |
| hepT      | Octaprenyl diphosphate synthase |          |
| fabG      | 3-oxoacyl-[acyl-carrier protein] reductase |          |
| CrtEb     | Lycopene elongase |          |
| crtB      | Phytoene synthase |          |
| Idi       | Isopentenyl-diphosphate delta-isomerase short-chain dehydrogenase/reductase SDR | Membrane fluidity |
| fabG      | 1-acyl-sn-glycerol-3-phosphate acyltransferase |          |
| aas       | 1-acyl-sn-glycerol-3-phosphate acyltransferase |          |
| Gds       | Geranylgeranyl diphosphate synthase |          |
| fabH      | 3-oxoacyl-[ACP] synthase III in alkane synthesis cluster |          |
| fabF      | 3-oxoacyl-[acyl-carrier-protein] synthase, KASII |          |
| plsC      | 1-acyl-sn-glycerol-3-phosphate acyltransferase |          |
| pcaH      | Protocatechuate 3,4-dioxygenase beta chain | Oxidative stress |
| pcaG      | Protocatechuate 3,4-dioxygenase alpha chain |          |
| trxC      | Thiosulfate sulfurtransferase, rhodanese |          |
| ntcA      | Transcriptional regulator, Crp/Fnr family |          |
| yrkH      | Hydroxacyclglutathione hydrolase |          |
| sodC      | Superoxide dismutase [Cu-Zn] precursor |          |
| Cob       | NAD-dependent protein deacetylase of SIR2 family |          |
| —         | Glutathione S-transferase domain protein |          |
| hcaC      | Ferredoxin, 2Fe-2S |          |
| soda      | Superoxide dismutase [Mn] |          |
| Fur       | Zinc uptake regulation protein ZUR |          |
| Gap       | glyceraldehyde-3-phosphate dehydrogenase |          |
| Gene Name | Gene Products | Function |
|-----------|---------------|----------|
| kata      | Catalase      |          |
|           | Glutaredoxin-like protein NrdH, required for reduction of Ribonucleotide reductase class Ib |          |
| nrdH      | Thioredoxin   |          |
| trxA      | Thioredoxin reductase |          |
| trxB      | Gamma-glutamyltranspeptidase |          |
| capD      | Lactoylglutathione lyase and related lyases |          |
| msrA      | Peptide methionine sulfoxide reductase |          |
| Dps       | Ferrooxidase  |          |
| yeaX      | Vanillate O-demethylase oxidoreductase |          |
| Line      | Glyoxalase family protein |          |
| Ohr       | Organic hydroperoxide resistance protein |          |
| rsME      | Ribosomal RNA small subunit |          |
| ywrD      | Thiol oxidoreductase |          |
| ahpC      | Alkyl hydroperoxide reductase subunit |          |
| Bcp       | C-type protein |          |
| trxB      | Thioredoxin reductase |          |
| pcaR      | Alkyl hydroperoxide reductase subunit |          |
| cobB1     | NAD-dependent protein deacetylase of SIR2 family |          |
| ntcA      | Transcriptional regulator, Crp/Fnr family |          |
| bphC      | Catechol 1,2-dioxidogenase |          |
| bphG      | 3- phenylpropionate dioxygenase |          |
| betP      | High-affinity choline uptake protein |          |
| gltB      | Glutamate synthase [NADPH] large chain |          |
| betC      | Choline-sulfatase |          |
| opuD      | Glycine betaine transporter |          |
| opuCA     | L-proline glycine betaine ABC transport system permease protein Prv |          |
| otsB      | Trehalose-6-phosphate phosphatase |          |
| proW      | L-proline glycine betaine ABC transport system permease protein |          |
| tcrY      | Osmosensitive K+ channel histidine kinase KdpD |          |
| otsA      | Alpha, alpha-trehalose-phosphate synthase [UDP-forming] |          |
| -         | Na(+) H(+) antiporter subunit G |          |
| -         | Na(+) H(+) antiporter subunit F |          |
| mrpD      | Na(+) H(+) antiporter subunit D |          |
### Table 3. Cont.

| Gene   | Gene Name      | Gene Products                                      | Function          |
|--------|----------------|----------------------------------------------------|-------------------|
|        | betA           | Choline dehydrogenase                              |                   |
|        | mrpE           | Na(+) H(+) antiporter subunit E                    |                   |
|        | minhC1         | Na(+) H(+) antiporter subunit C                    |                   |
|        | mrpA           | Na(+) H(+) antiporter subunit A; Na(+) H(+) antiporter subunit B |                   |
|        | opuCB          | Glycine betaine ABC transport system permease protein |                   |
|        | mrpG           | Na(+) H(+) antiporter subunit G                    |                   |
|        | mrpC           | Na(+) H(+) antiporter subunit C                    |                   |
|        |                | FIG152265: Sodium:solute symporter associated protein |                   |
|        |                | Na(+) H(+) antiporter subunit F                    |                   |
|        |                | Na(+) H(+) antiporter subunit E                    |                   |
|        | mrpD           | Na(+) H(+) antiporter subunit D                    |                   |
|        | betT           | High-affinity choline uptake protein               |                   |
|        | gltB           | Glutamate synthase [NADPH] small chain             | Osmo-protection   |
|        | ectA           | L-2,4-diaminobutyric acid acetyltransferase        |                   |
|        | gbsA           | Betaine aldehyde dehydrogenase                     |                   |
|        | betA           | Choline dehydrogenase                              |                   |
|        | baeS           | Osmosensitive K+ channel histidine kinase KdpD     |                   |
|        |                | Glutamate synthase [NADPH] large chain             |                   |
|        | gltB           | Glutamate synthase [NADPH] small chain             |                   |
|        | opuBB          | Glycine betaine ABC transport system permease protein |                   |
|        | putA           | Proline dehydrogenase (Proline oxidase)           |                   |
|        | ectC           | L-ectoine synthase                                 |                   |
|        | ectB           | Diaminobutyrate-pyruvate aminotransferase          |                   |
|        | panF           | Sodium:solute symporter, putative                 |                   |
|        | treS           | Trehalose synthase                                 |                   |
|        | osmF           | L-proline glycine betaine binding ABC transporter protein ProX |                   |
|        |                | Universal stress protein                          | General stress    |
|        |                | Serine phosphatase RsbU, regulator of sigma subunit |                   |
|        | glibO          | Hemoglobin-like protein HbO                        |                   |
|        | rpoE           | RNA polymerase sigma-70 factor, ECF subfamily       |                   |

#### 3.6.1. Cold Stress Response

Cold shock proteins are vital for the cold acclimation of bacteria [38]. Cold shock proteins (Csp) serve as nucleic acid chaperons, which counteract the harmful effects of cold stress like inefficient protein folding by regulating transcription and translation at low temperatures [39,40]. Csp have also been known to contribute to various environmental stress tolerance such as osmotic, oxidative, starvation and pH stress. PF2B19 genome contains genes encoding the cold shock proteins CspA and CspC and an arsenal of chaperones like dnaJ, dnaK and grpE, which are considered pivotal for preserving the integrity and function of proteins [41]. The genome also contains genes encoding the secondary CSPs polyribonucleotide nucleotidyltransferase (PNPase), ribosome binding factor A (RbfA), transcription elongation protein (NusA), and translation initiation factor (Inf2) which are typically induced via transcription anti-termination [42].
Modulation of membrane fluidity is crucial for cell viability at lower temperatures. This is achieved by improved production of unsaturated fatty acids, alteration of fatty acid branched chains and shortening of fatty acyl chains [43–45]. The Nesterenkonia sp. PF2B19 genome encodes five proteins involved in fatty acid biosynthetic pathways (Table 3). These include FabG and FabH involved in fatty acid biosynthesis, the condensation of fatty acids and the synthesis of branched fatty acids [35,46]. The genome also codes for 1-acyl-sn-glycerol-3-phosphate acyltransferase (PlsC), catalyzing the phospholipid synthesis, and 3-ketoacyl-(acyl-carrier-protein) reductase, involved in enhancing the production of polyunsaturated lipids [35,46]. Additionally, the pathway for unsaturated fatty acid synthesis was detected in PF2B19 using KEGG pathway tool.

At low temperatures, pigments are also known to modulate membrane fluidity [47–49]. The genome of PF2B19 contains three genes with putative roles in carotenoid biosynthesis (Table 3).

3.6.2. Oxidative Stress Response

Bacteria-harboring cold environments are more inclined to the deleterious effects of reactive oxygen species (ROS) because of better solubility of gases at low temperatures [45,50]. Nesterenkonia sp. PF2B19 encoded genes involved in detoxification of ROS such as catalase (kat), two superoxide dismutases (SodA; SodC), a thiol peroxidase (Bcp) as well as thioredoxin and thioredoxin reductase (TrxA and TrxB) [51]. Two putative dioxygenases were also detected in PF2B19 genome, known to play a key role in combating ROS damage [34].

3.6.3. Osmo-Protection

Accumulation of compatible solutes is an effective tactic to combat osmotic stress. These solutes are known to have dual response in stress as osmolytes and cryo-protectants [52]. Nesterenkonia sp. PF2B19 genome encodes a range of proteins involved in combating osmotic stress (Table 2). The genome also encodes transporters for glycine/betaine and choline dehydrogenases which are well-known osmo-protectants [53]. A number of genes involved in the endogenous synthesis of compatible solutes like trehalose biosynthesis genes otsA and otsB, known to be cold-inducible and essential for low temperature survival, were also detected.

3.6.4. General Stress Response

In addition to cold, osmotic and oxidative stress response, the PF2B19 genome encoded a repertoire of other stress-related proteins, which was included in general stress response system (Table 2). Ten genes involved in SOS response (cellular response to DNA damage) and DNA repair systems were detected. The genome also encoded universal stress protein, UspA, which is associated with cold acclimation [54].

3.7. Biotechnological Potential of PF2B19

Cold-active enzymes and the microbes producing them are of great biotechnological potential, with applications in detergent-, food-, textile-industry, pharmaceuticals and molecular biology. Psychrophilic enzymes are considered to be a boon to industry because of shorter process intervals, low energy budgets, low enzyme concentration requirement as well as impeding undesired chemical alterations [55]. Annotated genome sequence of PF2B19 revealed the presence of genes involved in production of cold-active enzymes, particularly of α-amylases, proteases, lipases/esterases, β-glucosidase, β-galactosidase and alkaline phosphatase (Table 4).
Furthermore, genes possibly responsible for hydrocarbon degradation were detected. Genes encoding catabolism of benzoate, catechol were found in the genome. Catechol compounds are the common intermediates in aerobic bacterial aromatic compound degradation pathways [56] and extradiol dioxygenases (EDOs) are known to catalyze the ring cleavage of catecholic compounds. EDOs like catechol 2,3-dioxygenase (EC 1.13.11.2), possible dioxygenase and 3-phenylpropionate dioxygenase ferredoxin subunit were detected in PF2B19. Benzoate catabolism genes 2-oxo-hepta-3-ene-1, 7-dioic acid hydratase (EC 4.2.-.-), and benzoate transport protein, 4-hydroxybenzoate transporter were also detected. Presence of these genes highlighted the bioremediation potential of PF2B19 in cold environment. Additionally, the pathway for degradation of catechol was elucidated in PF2B19 using KEGG database (Figure 3).

Table 4. PF2B19 genome-derived cold-adapted enzymes with their biotechnological applications.

| Cold-Active Enzymes Detected in PF2B19 Genome | Applications |
|-----------------------------------------------|--------------|
| Lipase, protease, phytase, xylanase            | Improves digestibility and assimilation of animal feed |
| Chitinase, protease                           | Meat tenderizing |
| α-amylase, xylanase                           | Textile industry |
| Esterase                                      | Chiral resolution of drugs to escalate effectiveness and range |
| β-lactamase                                   | Antibiotic degradation |
| Lipase                                        | Cosmetics, detergents |
| Chitinase                                     | Anti-fungal drug |
| β-galactosidase                               | Bioethanol production from dairy waste, improves the digestibility of dairy products for lactose-intolerant consumers |
| β-glucosidase                                 | Wine industry |
| Xylanase                                      | Biobleaching in paper and pulp industry |
| Lipase                                        | Biodiesel production by trans-esterification of oils and alcohols |
| Alkaline phosphatase                          | Cloning experiments in molecular biology |

PF2B19 also possessed the ability to promote plant growth. Genes involved in acetoin production, i.e., acetolactate synthase and zinc-containing alcohol dehydrogenase were identified in the genome. Acetoin is known to promote plant growth by stimulating root formation [57]. 1-aminoacyclopropane-1-carboxylate (ACC) deaminase gene (acdS) was also detected. acdS is known to aid the degradation of a plant’s ethylene precursor, thus promoting plant growth [58]. Arctic plants are challenged by various abiotic stressors in
their environment, which are known to limit their growth. PF2B19 can form mutualistic relationship with plants growing in the Arctic and promote growth. Moreover, the genes encoding proteins involved in resistance to heavy metals and toxic compounds (copper, cobalt, zinc, cadmium, mercury, chromium and arsenic) were detected in PF2B19, highlighting the potential of the PF2B19 to adapt to extreme lifestyles.

4. Conclusions

Based on genomic analysis, it can be concluded that Nesterenkonia sp. PF2B19 employs was found to be well-equipped with proteins involved in cold stress as well as modulation of membrane fluidity, osmotic and oxidative stress responses. Nesterenkonia sp. PF2B19 was found to be non-virulent and non-pathogenic. Genomic analysis of the PF2B19 has given valuable insight into the potential role of this strain in bioremediation in a colder environment. The genomic attributes also revealed the strategies adopted by Nesterenkonia sp. PF2B19 to survive in the extreme cold environment of permafrost.

Author Contributions: Conceptualization, formal analyses and methodology by P.S., N.K., V.G. Writing original draft preparation by P.S. and N.K. Resources and Supervision by P.K.D., S.M.S., M.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Science and Engineering Research Board (SERB), Grant No. (PDF/2016/003707), India.

Institutional Review Board Statement: This study did not involve humans or animals. Therefore, there are no ethical and biosafety issues.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data present in this research article will be available for public interest.

Acknowledgments: We are thankful to the Director, ARI for facilities. Purnima Singh is thankful to SERB-DST for financial support (PDF/2016/003707). Neelam Kapse is thankful to CSIR for the financial support (09/670 (0072)/2016-EMR-I). Thanks to the Almighty for driving us to complete this work during the difficult time of COVID-19.

Conflicts of Interest: The authors declare no conflict of interest.

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