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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

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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 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-adaption 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)

(http://www.genome.ad.jp accessed on 3 March 2021) database. Virulence determinants were detected using the online tool Virulence Finder [24].

2.5. Accession Nnumber

The *Nesterenkonia* sp. PF2B19 whole Genome Shotgun project has been deposited at GenBank under the accession no. MDSS00000000.

3. Results and Discussion

3.1. Characterization and Phylogeny of PF2B19

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).

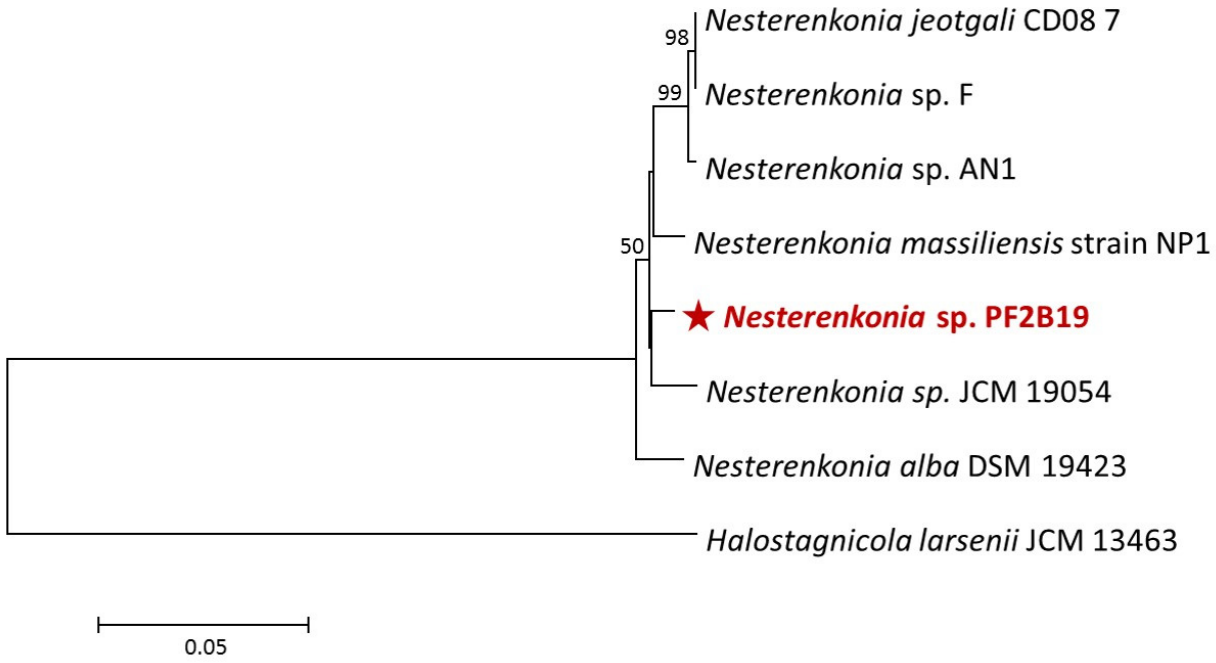

Figure 1. Phylogenetic tree displaying the taxonomic relationship between PF2B19 and other related members of the genus *Nesterenkonia.* (*Halostagnicola larsenii* JCM 13463 was used as an outgroup).

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 pf 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.

Table 1. Differentiating attributes between PF2B19 and publicly available other *Nesterenkonia* genomes.

Attributes	Strains of the Genus *Nesterenkonia* *						
	PF2B19	**CD08_7**	**AN1**	**F**	**JCM 19054**	**NP1**	**DSM 19423**
Accession no.	MDSS00000000	LQBM00000000	JEMO00000000	AFRW00000000	BAXI00000000	CBLL00000000	ATXP00000000
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.

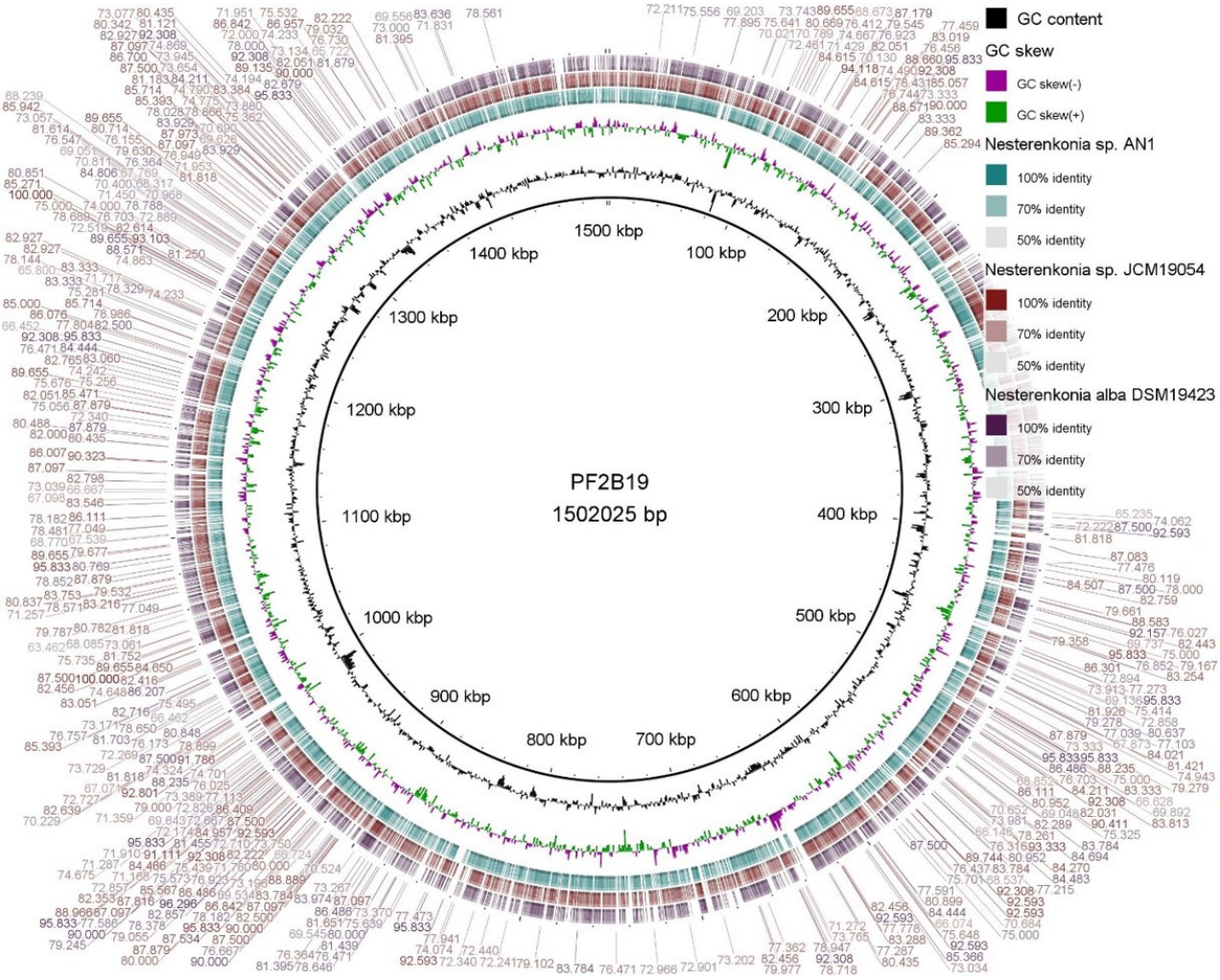

Figure 2. Circular map generated using BRIG program highlighting the differences between PF2B19 and publicly available *Nesterenkonia* genomes.

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	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. 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) 6. Ectoine hydroxylase (EC 1.17.-.-)	Counteract against cold-induced osmotic stress
		1. Glutathione synthetase (EC 6.3.2.3) 2. Hydroxy acyl glutathione hydrolase (EC 3.1.2.6) 3. Lactoylglutathione lyase (EC 4.4.1.5) 4. Redox-sensitive transcriptional activator SoxR 5. Transcriptional regulator, Crp/Fnr family	Counteract against cold-induced oxidative stress
		1. C50 carotenoid epsilon cyclase 2. Lycopene elongase (EC 2.5.1.-) 3. Phytoene dehydrogenase (EC 1.14.99.-) 4. Phytoene synthase (EC 2.5.1.32)	Modulate membrane fluidity at low temperatures
Nesterenkonia massilensis NP1	310	1. Starvation sensing protein RspA	Carbon Starvation
		1. Choline-sulfatase (EC 3.1.6.6) 2. Glycine betaine transporter OpuD 3. Sarcosine oxidase alpha, beta and gamma subunit (EC 1.5.3.1) 4. Ectoine hydroxylase (EC 1.17.-.-) 5. Outer membrane protein A precursor	Counteract against cold-induced osmotic stress
		1. Glutathione synthetase (EC 6.3.2.3) 2. Glutathione S-transferase, omega (EC 2.5.1.18) 3. Lactoylglutathione lyase (EC 4.4.1.5) 4. Redox-sensitive transcriptional activator SoxR	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. Sarcosine oxidase alpha, beta and gamma subunit (EC 1.5.3.1) 2. Outer membrane protein A precursor	Counteract against cold-induced osmotic stress
		1. Redox-sensitive transcriptional activator SoxR	Counteract against cold-induced oxidative stress
		1. Starvation sensing protein RspA	Carbon starvation
Nesterenkonia sp. AN1	202	1. Sarcosine oxidase alpha, beta and gamma subunit (EC 1.5.3.1) 2. Ectoine hydroxylase (EC 1.17.-.-) 3. Outer membrane protein A precursor	Counteract against cold-induced osmotic stress
		1. Redox-sensitive transcriptional activator SoxR	Counteract against cold-induced oxidative stress
		1. Starvation sensing protein RspA	Carbon starvation

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. JCM 19054	345	1. Cold shock protein CspA	Cold shock response
		1. Glycine betaine transporter OpuD 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	Counteract against cold-induced osmotic stress
		1. Lactoylglutathione lyase (EC 4.4.1.5) 2. Redox-sensitive transcriptional activator SoxR 3. Transcriptional regulator, FUR family 4. Transcriptional regulator, Crp/Fnr family	Counteract against cold-induced oxidative stress
		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).

Table 3. Cold-induced stress associated genes in *Nesterenkonia* sp. PF2B19 genome.

Gene Name	Gene Products	Function
cshA	Putative ATP-dependent RNA helicase	**Cold stress**
cspC	Cold shock protein C	
cspA	Cold shock protein A	
infB	Translation initiation factor 1	
deaD	DEAD-box ATP-dependent RNA helicase CshA	
Pnp	Polyribonucleotide nucleotidyl transferase	
infB	Translation initiation factor 2	
rbfA	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. *Cont.*

Gene Name	Gene Products	Function
recA	Recombinase	**DNA repair**
recN	DNA repair protein	
recR	Recombination protein	
uvrA	Excinuclease ABC subunit A paralog of unknown function	
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	
fabG	short-chain dehydrogenase/reductase SDR	
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	
	Lactoylglutathione lyase	
yrkH	Hydroxyacylglutathione 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	NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase	

Table 3. *Cont.*

Gene Name	Gene Products	Function
kata	Catalase	
nrdH	Glutaredoxin-like protein NrdH, required for reduction of Ribonucleotide reductase class Ib	
trxA	Thioredoxin	
trxB	Thioredoxin reductase	
capD	Gamma-glutamyltranspeptidase	
_	Lactoylglutathione lyase and related lyases	
msrA	Peptide methionine sulfoxide reductase	
Dps	Ferroxidase	
yeaX	Vanillate O-demethylase oxidoreductase	
Line	Glyoxalase family protein	
Ohr	Organic hydroperoxide resistance protein	
rsmE	Ribosomal RNA small subunit methyltransferase E	
ywrD	Gamma-glutamyltranspeptidase	
ahpC	Alkyl hydroperoxide reductase subunit C-like protein	
Bcp	Thiol peroxidase, Bcp-type	
trxB	Thioredoxin reductase	
pncB1	Nicotinate phosphoribosyltransferase	
Fur	Transcriptional regulator, FUR family	
hcaC	3-phenylpropionate dioxygenase ferredoxin subunit	
bphG	Ferredoxin reductase	
pcaR	Transcriptional regulator, IclR family	
cobB1	NAD-dependent protein deacetylase of SIR2 family	
ntcA	Transcriptional regulator, Crp/Fnr family	
bphC	Catechol 2,3-dioxygenase	
bphG	3-phenylpropionate dioxygenase ferredoxin subunit	
	Nicotinamidase	
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 ProV	
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 Name	Gene Products	Function
betA	Choline dehydrogenase	
mrpE	Na(+) H(+) antiporter subunit E	
mnhC1	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	
ectA	L-2,4-diaminobutyric acid acetyltransferase	**Osmo-protection**
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	
-	Serine phosphatase RsbU, regulator of sigma subunit	**General stress**
glbO	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 (Csps) 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]. Csps 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).

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	Additive for anti-fungal creams and lotions, 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

Furthermore, genes possibly responsible for hydrocarbon degradation were detected. Genes encoding catabolism of benzoate, catechol were found in the genome. Catecholic compounds are the common inter-mediates 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).

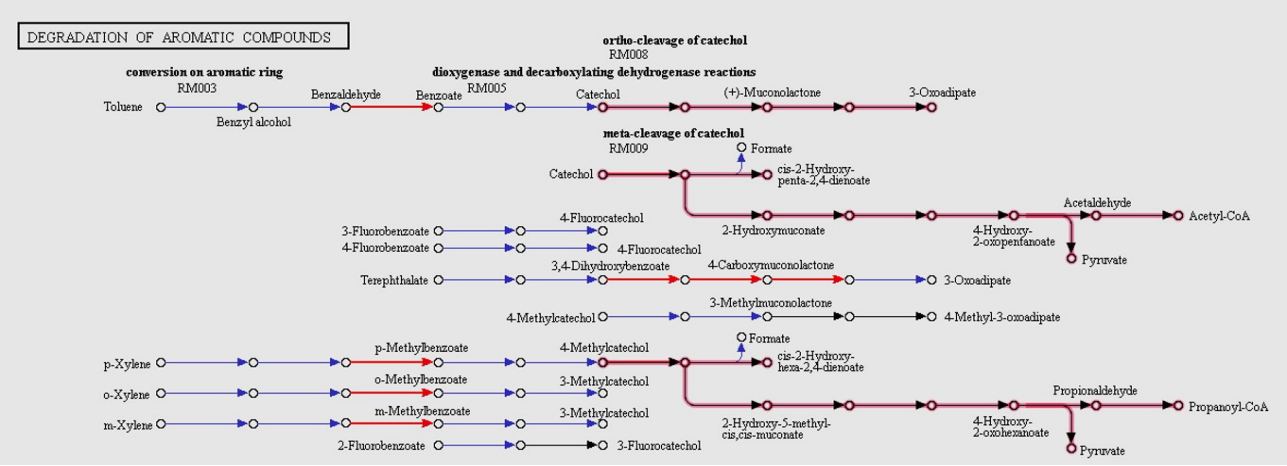

Figure 3. Pathway for degradation of catechol was elucidated using KEGG server.

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-aminocyclopropane-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.

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