Complete genome of *Arthrobacter alpinus* strain R3.8, bioremediation potential unraveled with genomic analysis

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**Abstract**

*Arthrobacter alpinus* R3.8 is a psychrotolerant bacterial strain isolated from a soil sample obtained at Rothera Point, Adelaide Island, close to the Antarctic Peninsula. Strain R3.8 was sequenced in order to help discover potential cold active enzymes with biotechnological applications. Genome analysis identified various cold adaptation genes including some coding for anti-freeze proteins and cold-shock proteins, genes involved in bioremediation of xenobiotic compounds including naphthalene, and genes with chitinolytic and N-acetylglucosamine utilization properties and also plant-growth-influencing properties. In this genome report, we present a complete genome sequence of *A. alpinus* strain R3.8 and its annotation data, which will facilitate exploitation of potential novel cold-active enzymes.

**Keywords:** Cold active enzymes, Anti-freeze protein, Chitinase, Psychrotolerant bacteria, Plant growth promoting bacteria

**Introduction**

The production of cold-adapted enzymes by psychrotolerant bacteria has important scientific and industrial interest due to their highly specific activity and catalytic efficiency at low and moderate temperatures [1]. The use of cold-adapted enzymes offers various advantages such as the reduction of undesirable chemical reactions that take place at high temperature, rapid enzymatic inactivation through thermal treatment, and reduction in energy demand required to fuel industrial processes at higher temperatures [2–4]. These beneficial traits are particularly useful in the development of sequential molecular biology processes, low temperature detergents, food and industrial bio-catalytic enzymes, and for bioremediation agents applicable during cold seasons and in cold regions. In this study, we perform complete genome sequencing on a psychrotolerant bacterium, *Arthrobacter alpinus* strain R3.8 (DSM 100969), originally isolated from soil collected from Rothera Point, Adelaide Island, maritime Antarctica. The optimum growth temperature range of this bacterium is 10–16 °C, which rendered it a promising source for discovery of novel cold-adapted enzymes. The complete genome sequence of *A. alpinus* strain R3.8 was generated using Single Molecule Real Time sequencing technology to provide a rapid and complete insight into its biotechnological potential. Here, we highlight various genome features that indicate the potential biotechnological value of *A. alpinus* strain R3.8 in the context of xenobiotic biodegradation and metabolism, chitin utilization, and as a potential component in bio-fertilizers.

**Organism information**

**Classification and features**

*A. alpinus* strain R3.8, is a psychrotolerant soil bacterium originally isolated from a soil sample collected at Rothera Research Station, close to Antarctic Special Protected Area No.129 (68°07′S, 67°34′W). Strain R3.8 was isolated using basal medium supplied with C6-HSL as sole carbon source. An isolation temperature of 4 °C was used to select for psychrophilic or psychrotolerant bacteria maintained on Luria Bertani (LB) agar [5, 6]. The strain exhibited a 98.6% 16S rRNA nucleotide
sequence similarity with *A. alpinus*, the most phylogenetically closely related *Arthrobacter* species with standing in nomenclature (Fig. 1). The cells are Gram-positive, coccoid, and approximately 2.0 μM in width and 1.8 μM in length (Fig. 2). This pairwise 16S rRNA gene sequence similarity value suggested that strain R3.8 is *A. alpinus*, following the species delineation threshold recommended by Stackebrandt and Ebers [7]. API test strips (API 20 E, API 20 E and API ZYM) incubated at 20 °C were used according to the manufacturer’s instructions to determine the physiological and biochemical characteristics as well as enzyme activities of strain R3.8. The results were compared with type strain of *A. alpinus* strain S6-3T. Strain R3.8 showed a closely similar biochemical profile with S6-3T in all the API tests. Both strains did not produce catalase and cytochrome oxidase and were able to hydrolyze aesculin. Both strains were positive for activities of acidic phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase and α-mannosidase, and could utilize D-glucose, lactose, L-arabinose, maltose, D-mannose, D-mannitol and N-acetylglucosamine as sole carbon source. Both strains were negative in indole production, H₂S production and citrate utilization. Both were also negative for activities of arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, lipase (C14), N-acetyl-β-glucosaminidase, trypsin, α-chymotrypsin, and α-fucosidase, and negative for the fermentation of glucose, mannitol, sucrose, inositol, sorbitol, rhamnose, melibiose, and amygdalin. However, strain R3.8 was not able to hydrolyze urea, unlike strain S6-3T, in both API 20 E and API 20 NE tests. In the API
20 NE test, strain R3.8 was positive for nitrate reduction, differing from strain S6-3\textsuperscript{T}. In the API 20 E test, strain R3.8 was positive for fermentation of L-arabinose but strain S6-3\textsuperscript{T} was negative. In the API ZYM test, strain R3.8 did not produce alkaline phosphatase and naphthol-AS-BI-phosphohydrolase as produced by strain S6-3\textsuperscript{T}.

Minimum Information about the Genome Sequence of \textit{A. alpinus} strain R3.8 is summarized in Table 1.

**Genome sequencing information**

**Genome project history**

The genome of \textit{A. alpinus} strain R3.8 was sequenced to study its bioremediation properties, specifically focusing on naphthalene biodegradation. The assembled and annotated genome of \textit{A. alpinus} strain R3.8 described in this paper has been deposited in GenBank (accession number of CP12677.1), the KEGG database (entry number of T04095) and the JGI portal with GOLD ID of Gp0124186 and IMG taxon ID of 2645727552. Sequencing, assembly and annotation of the complete genome were performed by the UM Omics Centre, University of Malaya, Malaysia. A summary of the project information is shown in Table 2.

**Growth conditions and genomic DNA preparation**

\textit{A. alpinus} strain R3.8 was grown aerobically in 5.0 ml LB broth at 16 °C. A volume of 1.0 ml was then centrifuged at 2500 x g for 5 min at 4 °C and genomic DNA was extracted and purified using the MasterPure\textsuperscript{TM} DNA purification kit (Epicenter Technologies, USA) following the manufacturer’s instructions. The purity and quality of the genomic DNA obtained were assessed using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, USA) and quantified using Qubit 2.0 fluorometer (Life Technologies, MA, USA).

**Genome Sequencing and Assembly**

The sheared genomic DNA of \textit{A. alpinus} strain R3.8 was constructed into a 20 kb SMRTbell template library following the ‘Procedure and Checklist - 20 kb Template Preparation Using BluePippin’ Size-Selection System’ protocol [8, 9]. The purified and size-selected SMRTbell library was sequenced in five SMRT cells using P6C4 chemistry on a PacBio RS II sequencing system (Pacific Biosciences, USA). Sub-reads generated from the raw sequencing reads following adapter-removal were used as input data for de novo assembly using Hierarchical Genome Assembly Process version 2 [10]. The assembly of the \textit{A. alpinus} strain R3.8 genome was based on 64,388 quality reads with a mean length of 7,335 bp resulting in a single circular chromosome consisting of 4,046,453 bp with 101.74-fold overall coverage.

| MIGS ID | Property | Term | Evidence code\(a\) |
|---------|----------|------|---------------------|
| Current classification | Domain Bacteria | TAS | \[35\] |
| | Phylum Actinobacteria | TAS | \[36\] |
| | Class Actinobacteria | TAS | \[37\] |
| | Subclass Actinobacteridae | TAS | \[37–38\] |
| | Order Actinomycetales | TAS | \[37–40\] |
| | Family Micrococccaceae | TAS | \[37–39, 41\] |
| | Genus Arthrobacter | TAS | \[39, 42, 43\] |
| | Species Arthrobacter alpinus | TAS | \[42\] |
| | Strain R3.8 | IDA | \[\] |
| Gram stain | Positive | TAS | \[42\] |
| Cell Shape | irregular rods, coccoid | IDA | \[\] |
| Motility | Non-motile | TAS | \[42\] |
| Sporulation | Non-sporulating | TAS | \[42\] |
| Temperature range | 4–30 °C | IDA | \[\] |
| Optimum temperature | 20–25 °C | IDA | \[\] |
| pH range; Optimum | 6.0–9.0;7.0 | IDA | \[\] |
| Carbon source | Acyl-homoserine lactone (AHLs), Yeast extract | IDA | \[\] |
| MIGS-6 | Habitat | soil | IDA |
| MIGS-6.3 | Salinity | up to 10% NaCl (w/v) | IDA |
| MIGS-22 | Oxygen requirement | Aerobic | TAS | \[42\] |
| MIGS-15 | Biotic relationship | Free living | NAS |
| MIGS-14 | Pathogenicity | Non-pathogen | NAS |
| MIGS-4 | Geographic location | Northern end of Rothera Point, adjacent to Antarctic Specially Protected Area 129, Antarctica | NAS |
| MIGS-5 | Sample collection | December 2009 | NAS |
| MIGS-4.1 | Latitude | 68°07’S | NAS |
| MIGS-4.2 | Longitude | 67°34’W | NAS |
| MIGS-4.4 | Altitude | 33 m | NAS |

\(a\)Evidence codes – IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project

**Genome Annotation**

Gene prediction and annotation were performed using the Rapid Annotation Search Tool [11], Rapid Prokaryotic Genome Annotation [12] and NCBI Prokaryotic Genome Annotation Pipeline based on the best-placed reference protein set and GeneMarkS+. Additional gene identification was made using the KEGG database [13],
Carbohydrate-Active Enzymes Database ([14], Pathosystem Resource Integration Center [15], and IMG ER [16]).

Genome Properties
With 101.74 fold of coverage, the genome of *A. alpinus* strain R3.8 was assembled into a 4,046,453 bp circular chromosome with an average GC content of 62.2% (Table 3). No plasmid sequence was identified in this assembly (Table 1 and Fig. 3). A total of 3,697 genes was predicted of which 3,268 genes were identified as protein coding genes. A total of 69 RNA genes were also identified consisting of 18 rRNA (6 5S rRNA, 6 16S rRNA, and 6 23S rRNA) and 51 tRNA genes. 169 (5.17%) were designated as pseudo genes, 57 (1.74%) genes were frameshifted (Table 3). Furthermore, 61.54% of the predicted genes (3,892) are represented by COG functional categories. Distribution of these genes and their percentage representation are listed in Table 4. The genome sequence is deposited in GenBank (accession number CP12677.1), from which the genome sequence data can be accessed in the format of FASTA, annotated GenBank flat file, graphical and ASN.1 file.

Table 2 Project information

| MIGS ID | Property               | Term                                      |
|---------|------------------------|-------------------------------------------|
| MIGS 31 | Finishing quality      | Finished                                  |
| MIGS 28 | Libraries used         | One library, PacBio 20-kb SMRTbell Library |
| MIGS 29 | Sequencing platforms   | PacBio RS                                 |
| MIGS 31.2| Fold coverage         | 101.74X                                  |
| MIGS 30 | Assemblers             | HGAP v. 2.20.p1 [40]                     |
| MIGS 32 | Gene calling method    | Prodigal 1.4, GeneMark, Glimmer v. 2.13  |
| Locus Tag|                        | AOC05                                     |
| Genbank ID   |                        | CP012677.1                                |
| GenBank Release Date |                | 28-MARCH-2016                            |
| GOLD ID     |                        | Gp0124186                                 |
| BioProject ID |                    | PRNUA224116                               |
| MIGS 13 | Source Material Identifier | DMS 100969       |
| Project relevance |                  | Environmental, Biotechnological          |

Insights from the genome sequence
Functional annotation results of this genome are accessible from the complete genome directory of the KEGG ORGANISMS database with the organism prefix of aaq. Further, through the aaq hyperlink, cross-reference information is available in the form of protein, and small-molecules interaction network maps, BRITe biological systems hierarchical classifications, KEGG modules, and a whole genome map which can be visualized using genome map browser can be accessed through the subdirectory panel.

Cold-adaptation genes
An antifreeze protein [AOC05_08780], a gene encoding a protein with ice-nucleation activity reported to be secreted by psychrotolerant bacterium into the surrounding medium at low temperatures to prevent the formation of ice crystals [17–19], was identified in the genome. Various temperature stress response genes were also identified. For example, the cold shock protein family that has been shown to allow bacterial response to rapid temperature shift, allowing bacteria cells to function to survive above their thermal optimum by serving as nucleic acid chaperones that may prevent the formation of secondary structures in mRNA at low temperature [20]. The NCBI locus tags for the cold shock proteins that were identified are AOC05_RS02130, AOC05_13125, and AOC05_RS01570.

Biodegradation genes
Naphthalene is a group C (possible human carcinogen) benzenoid polycyclic aromatic hydrocarbon and is a pollutant widely encountered in nature [21, 22]. In 1990, naphthalene was recognized as one of the priority pollutants required to be controlled by the Environmental Protection Agency of the United States. In the genome of *A. alpinus* strain R3.8, various genes that are involved in naphthalene biodegradation were identified, including salicylate 1-monooxygenase [AOC05_08330], imidazole glycerol phosphate synthase cyclase [AOC05_05535] that is involved in 1- and 2-methyl naphthalene degradation, and other genes involved in 1,4-dichlorobenzene.

Table 3 Genome statistics of *Arthrobacter alpinus* R3.8

| Attribute                  | Value          | % of Total |
|----------------------------|----------------|------------|
| Genome size (bp)           | 4,046,453      | 100        |
| DNA coding (bp)            | 3,578,529      | 88.44      |
| DNA G + C (bp)             | 2,516,755      | 62.20      |
| DNA scaffolds              | 1              | 100        |
| Total genes                | 3892           | 100        |
| Protein coding genes       | 3817           | 98.07      |
| RNA genes                  | 75             | 1.93       |
| Pseudo gene                | 359            | 9.71       |
| Genes in internal cluster  | 848            | 21.79      |
| Genes with functional prediction | 2870          | 73.74      |
| Genes assigned to COGs     | 2395           | 61.54      |
| Genes with Pfam domains    | 2987           | 76.75      |
| Genes with signal peptides | 101            | 2.60       |
| Genes with transmembrane helices | 942      | 24.20      |
| CRISPR repeat              | 1              |            |
degradation, namely enoyl-CoA hydratase [AOC05_00980, AOC05_05365, AOC05_11215, AOC05_11270, AOC05_11290, AOC05_11310, AOC05_13700, AOC05_13710], alkaline phosphatase [AOC05_01425, AOC05_03310, AOC05_12520], nitrilotriacetate monooxygenase [AOC05_10370] and aliphatic amidase amiE [AOC05_16895].

Furthermore, two genes involved in the production of urease were also identified in the genome of A. alpinus strain R3.8, urease alpha subunit [AOC05_06080] and urease gamma subunit [AOC05_18490]. Urease is important in catalyzing one of the metabolic pathways involved in microbial-induced calcite precipitation. MICP is a promising approach in the containment of heavy metals such as lead and cadmium in contaminated soils [23, 24].

Various other xenobiotic biodegradation genes and pathways of A. alpinus strain R3.8 are available from the PATRIC server.

Genes with chitinolytic and N-acetylglucosamine utilization properties

Chitinase is a biotechnologically-important enzyme widely used in waste management industries for the degradation of chitinous waste into simpler depolymerized substances [25], in agricultural industries for engineering of transgenic crops with resistance to fungal infection [26] and in healthcare industries for the therapeutic treatment of fungal infections [25, 27]. A range of recent studies have identified and characterized novel cold-active chitinase enzymes with higher catalytic efficiency at low temperatures [28–31].

The full chitinolytic potential of A. alpinus strain R3.8 was also identified here, with various genes involved in chitin and N-acetylglucosamine utilization being identified, including beta-hexosaminidase (EC 3.2.1.14) [AOC05_06080], eukaryotic type N-acetylglucosamine kinase (EC 2.7.1.59) [AOC05_12050], PTS system, N-acetylglucosamine (EC 2.7.1.59) [AOC05_12345], N-acetylglucosamine-6-phosphate deacylase (EC 3.5.1.25) [AOC05_15120], eukaryotic type N-acetylglucosamine kinase (EC 2.7.1.59) [AOC05_15120], chitinase (EC 3.2.1.14) [AOC05_02965], N-acetyl-glucosamine kinase 2, ROK family (EC 2.7.1.59) [AOC05_03295], transcriptional regulator of N-acetylglucosamine utilization, GntR family [AOC05_07215] and glucosamine-6-phosphate deaminase (EC 3.5.99.6) [AOC05_10045].

Potential plant growth promoting properties

Application of psychrotrophic PGP bacteria to vegetation can promote growth and improve cold tolerance of crops [32]. From the RAST analysis, a total of 22 PGP genes were identified in the genome of A. alpinus strain

![Fig. 3](image_url)
Table 4 Number of genes associated with general COG functional categories

| Code | Value | % age | Description |
|------|-------|-------|-------------|
| J    | 180   | 6.69  | Translation, ribosomal structure and biogenesis |
| A    | 1     | 0.04  | RNA processing and modification |
| K    | 228   | 8.48  | Transcription |
| L    | 112   | 4.17  | Replication, recombination and repair |
| B    | 1     | 0.04  | Chromatin structure and dynamics |
| D    | 25    | 0.93  | Cell cycle control, Cell division, chromosome partitioning |
| V    | 53    | 1.97  | Defense mechanisms |
| T    | 113   | 4.2   | Signal transduction mechanisms |
| M    | 106   | 3.94  | Cell wall/membrane biogenesis |
| N    | 28    | 1.04  | Cell motility |
| Z    | 1     | 0.04  | Cytoskeleton |
| W    | 3     | 0.11  | Extracellular structures |
| U    | 25    | 0.93  | Intracellular trafficking and secretion |
| O    | 104   | 3.87  | Posttranslational modification, protein turnover, chaperones |
| C    | 183   | 6.81  | Energy production and conversion |
| G    | 242   | 9     | Carbohydrate transport and metabolism |
| E    | 281   | 10.45 | Amino acid transport and metabolism |
| F    | 80    | 2.98  | Nucleotide transport and metabolism |
| H    | 156   | 5.6   | Coenzyme transport and metabolism |
| I    | 151   | 5.62  | Lipid transport and metabolism |
| P    | 174   | 6.47  | Inorganic ion transport and metabolism |
| Q    | 74    | 2.75  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 228   | 8.48  | General function prediction only |
| S    | 115   | 4.28  | Function unknown |
| -    | 1497  | 58.46 | Not in COGs |

The total is based on the total number of protein coding genes in the genome.

R3.8. The genes that are involved in the glutamine synthetase (GS)-glutamate synthase (GOGAT) pathway of bacterial ammonia assimilation are glutamine synthetase (GS) [AOC05_04790] and glutamate synthase (GOGAT) [AOC05_05585]. Full details of these genes, including nitrogen regulatory protein [AOC05_18400] and ammonium transporter [AOC05_09335] are available in Additional file 1: Table S1. Furthermore, five genes involved in plant hormone biosynthesis, indole acetic acid biosynthesis, including monoamine oxidase [AOC05_17530], tryptophan synthase [AOC05_05570, AOC05_05575], anthranilate phosphoribosyltransferase [AOC05_07190] and N-(5′-phosphoribosyl) anthranilate isomerase [AOC05_12350] were identified. Production of bacterial IAA is important to assist plants to overcome abiotic stresses and inhibitory compounds, and thus contributes to plant growth stimulation [33]. Several other PGP-relevant genes involved in trehalose synthesis [AOC05_15010, AOC05_00140, AOC05_00145, AOC05_00495, and AOC05_00500] and in spermidine synthesis [AOC05_16565] were also identified in the genome.

Conclusions

We report the complete genome sequence of *Arthrobacter alpinus* strain R3.8 that was originally isolated from the soil collected from Rothera Point, Adelaide Island, maritime Antarctica. The strain was sequenced to explore its biotechnological potential. By analyzing the complete genome of *A. alpinus* strain R3, we identified genes involved in xenobiotic biodegradation and metabolism, and chitin utilization, as well as genes that potentially promote plant growth. Further comparative genomic studies with related isolates together with functional studies will provide better understanding of the potential biotechnological value of this strain.

Additional file

Additional file 1: Table S1. Genes involved in chitin degradation identified from RAST analysis. (DOCX 18 kb)

Abbreviations

HGAP: Hierarchical Genome Assembly Process; IAA: Indole acetic acid; MICP: Microbial-induced calcite precipitation; MGIs: Minimum Information about the Genome Sequences; PATRIC: Pathosystem Resource Integration Center; PGP: Plant Growth Promoting; RAST: Rapid Prokaryotic Genome Annotation; SMRT: Single Molecule Real Time

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Authors’ contributions

WSST conceived and designed the experiments. WSST and YLL performed the sequencing and assembly experiments. WSST drafted the manuscript, with input and revisions from RE, YLL, PC, DP, TBMM, WFY and KGC. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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