Complete genome sequence of Halomonas sp. R5-57

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Abstract

The marine Arctic isolate Halomonas sp. R5-57 was sequenced as part of a bioprospecting project which aims to discover novel enzymes and organisms from low-temperature environments, with potential uses in biotechnological applications. Phenotypically, Halomonas sp. R5-57 exhibits high salt tolerance over a wide range of temperatures and has extra-cellular hydrolytic activities with several substrates, indicating it secretes enzymes which may function in high salinity conditions. Genome sequencing identified the genes involved in the biosynthesis of the osmoprotectant ectoine, which has applications in food processing and pharmacy, as well as those involved in production of polyhydroxyalkanoates, which can serve as precursors to bioplastics. The percentage identity of these biosynthetic genes from Halomonas sp. R5-57 and current production strains varies between 99% for some to 69% for others, thus it is plausible that R5-57 may have a different production capacity to currently used strains, or that in the case of PHAs, the properties of the final product may vary. Here we present the finished genome sequence (LN813019) of Halomonas sp. R5-57 which will facilitate exploitation of this bacterium; either as a whole-cell production host, or by recombinant expression of its individual enzymes.

Keywords: Halomonas, Growth temperature, Salt tolerance, Secreted enzymes, Osmolyte, Polyhydroxyalkanoates

Abbreviations: COG, Cluster of orthologous groups; PHAs, Polyhydroxyalkanoates; RDP, Ribosomal database project; SMRT, Single molecule real-time

Introduction

Halomonas sp. R5-57 is a marine member of the Halomonadaceae, a family of Gram-negative chemoorgano-trophic bacteria that display moderate to high salt tolerance. Members of this genus have been isolated from diverse saline environments such as ocean water [1, 2], salterns [3], marine hydrothermal vents [4], hypersaline lakes [5, 6] and salted fermented food [7]. Several species of Halomonas have also been identified as human pathogens [1, 8, 9]. To date draft genomes of 15 Halomonas species (H. zincidurans B6, H. halodenitrificans DSM 735, DSM 1457, H. lutea DSM 2350, H. antarciensis FP35 DSM 16096, H. zhanjiangensis DSM 2107, H. jeotgali Hwa, H. titanicae BH1, H. snyrensis AAD6, H. stevensii S18214, H. boliviensis LC1, H. casei- nitlytica ASM81542v1, H. hydrothermalis HaloHydro1.0, H. xinjiangensis ASM75934v1 and H. salina) and complete genomes of two species (H. elongata DSM 2581 ASM19687v1 and H. campaniensis ASM69648v1) are available.

Halomonas species have a number of technologically exploitable features. Both compatible solutes, which the bacteria accumulate as part of their adaptation to saline environments, and extracellular polymers, which protect the cells from environmental stresses and aid in biofilm formation, are used in pharmaceutical, food-processing and biotechnological industries [10, 11]. Additionally, polyhydroxyalkanoates which are accumulated by the bacterium as energy storage compounds can be used to produce biodegradable plastic materials [12]. Finally, the high solubility of Halomonas proteins, both in their folded and unfolded states have led to their use as fusion tags for improving the solubility of recombinantly expressed proteins [13].

The isolation, characterization and genome sequencing of Halomonas sp. R5-57 was undertaken as part of the
MARZymes project which aims to identify novel cold-adapted enzymes and organisms from marine sources. Here we present the complete genome sequence of *Halomonas* sp. R5-57 together with its temperature and salinity growth optima and functional screening for various activities.

**Organism information**

**Classification and features**

*Halomonas* sp. R5-57 was isolated from the skin of the red sea squirt *Halocynthia papillosa* collected from the Barents Sea in Spring 2009. The animal was dissected and the skin homogenized in an equal volume of sterile sea water and 50 μl was plated onto IM8 media [14]. An individual colony was picked from this raw plate after incubation at 4 °C for two weeks, and was subsequently re-streaked two times and grown at 4 °C for 1 week.

Liquid cultures for DNA isolation and growth curves were prepared by inoculating Luria-Bertani media with 3.5 % NaCl from these pure isolates. A summary of the isolation and phenotypic characteristics of *Halomonas* sp. R5-57 are given in Table 1.

PCR product of the partial 16S rRNA gene was generated using the 27F and 1492R universal primers [15], and then sequenced with the BigDye terminator kit version 3.1 (Applied Biosystems) using the 515 FD primer. This placed isolate R5-57 with other psychrotolerant species of *Halomonas*, having 99 % identity to *H. glaciei* DD 39T (MTCC 4321; JCM 11692), isolated from fast ice in Antarctica [16]. Neighbor-joining analysis of the full-length 16S rRNA gene shown in Fig. 1, separates *Halomonas* sp. R5-57 from the related *H. titanicae* BH1 (99.4 %), *H. boliviensis* (99.0 %) and *H. variabilis* DSM 3051 (99.5 %).

| MIGS ID | Property | Term | Evidence code |
|---------|----------|------|---------------|
| MIGS-6  | Habitat  | Marine Arctic | IDA |
| MIGS-6.3| Salinity | Requires >1 % NaCl, tolerates up to 12 % NaCl. Optimum is 3.5-7.0 % NaCl | IDA |
| MIGS-22 | Oxygen requirement | Aerobic | TAS [43] |
| MIGS-15 | Biotic relationship | Free living, isolated from the skin of the red sea squirt *Halocynthia papillosa* | NAS/IDA |
| MIGS-14 | Pathogenicity | Not reported | NAS |
| MIGS-4  | Geographic location | Sagaskjær | IDA |
| MIGS-5  | Sample collection | 14.05.2009 | IDA |
| MIGS-4.1| Latitude | 78.12.78372 N | IDA |
| MIGS-4.2| Longitude | 013.58.27000 E | IDA |
| MIGS-4.4| Altitude | −180.42 m | IDA |

* 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 [44].
Scanning electron micrographs show that this bacterium is rod-shaped and has a number of flagella with a peritrichous arrangement (Fig. 2). Cells for microscopy were taken from colonies after 24 h growth and fixed with 5 % glutaraldehyde for 1 h, then 2.5 % glutaraldehyde overnight. Fixed suspensions were applied to Poly-L-Lysine coated slides for 2–5 min and post-fixed with 1 % osmium tetroxide for 30 min followed by dehydration with increasing concentrations of ethanol (30 %, 60 %, 90 %, 96 %, 5 min each, 99 % 5 min twice) hexamethyldisilazane (2 min, two times), and finally incubation in a desiccator with silica gel for approximately 2 h. Dried specimens were sputter-coated with gold and observed with a ZEISS MERLIN Scanning Electron Microscope with an accelerating voltage of 2.0 kV.

Members of the Halomonadaceae are characterized by having high salt tolerance, and as the 16S rRNA sequence of Halomonas sp. R5-57 clusters with other psychrotolerant strains H. titanicae, H. variabilis and H. boliviensis, we investigated both the salinity and temperature optimum of this isolate. Growth rates measured on LB medium containing 0.5 - 12 % NaCl at temperatures between 4 – 41 °C show Halomonas sp. R5-57 has an optimum of 20 °C in 3.5 % NaCl, the salinity of seawater, and requires minimum salt concentration of 1.0 % for any significant growth to occur. The salinity of the medium also had a marked effect on the temperature tolerance of Halomonas sp. R5-57 as below 7 % NaCl growth rates peaked at 20 °C then decreased rapidly; but at 10 – 12 % NaCl the temperature optimum increased to 30 °C and growth was observed at up to 41 °C (Additional file 1: Figure S1).

Metabolic activities of Halomonas sp. R5-57 were determined with the API® system, using tests NE and E (bioMérieux). Tests were conducted at 25 °C, all media was supplemented with 3.5 % NaCl and final results were scored after 5 days. Halomonas sp. R5-57 is oxidase positive, reduced nitrate to nitrite, was able to utilize citrate, ferment or oxidize glucose, manitol, inositol, sorbitol, melibiose, saccharose, melibiose amygdaline arabinose, and assimilate N-acetyl glucosamine, potassium gluconate, capric acid and adipic acid. Additionally this strain displayed beta galactosidase, arginine dehydro-lase gelatinase activities, and hydrolysed esculin.

Substrate utilisation was also examined by plate-based screens conducted at 4 and 20 °C on marine broth supplemented with the following indicator substrates: 1.5 % w/v carboxymethylcellulose (cellulase); 0.1 % w/v sodium alginate (alginate lyase); 2 % w/v starch, then stained with 0.5 % Congo Red, 5 % ethanol (amylase); 2.5 g/L xylan (xylanase); 0.5 % w/v chitin (chitinase); 1 % w/v skimmed milk (protease), 0.4 % w/v gelatin then stained with Coo massie Blue G-250 (gelatinase); 1 % v/v tributyrin (lipase/esterase); or on LB media supplemented with 3.5 % NaCl and DNA (DNase). Results were recorded by the presence of a halo on the plate after 1 week, and revealed that Halomonas sp. R5-57 has secreted chitinase, DNase and protease activities at 20 °C, and lipase activity at 4 °C.
**Genome sequencing information**

**Genome project history**

*Halomonas* sp. R5-57 was selected for genome sequencing on the basis of its phylogenetic position that grouped this isolate with other psychrotolerant species of *Halomonas*. The project commenced with collection of the isolate in 2009, and Illumina sequencing was completed at the Norwegian Sequencing Centre in July 2012, followed by Pacific Biosciences (PacBio) sequencing in January 2015. The finished sequence of *Halomonas* sp. R5-57 was completed in February 2015 and deposited in the European Nucleotide Archive [17] with the identifier LN813019 (GI:802125597).

Table 2 presents the project information and its association with MIGS version 2.0 compliance [18].

**Growth conditions and genomic DNA preparation**

Pure cultures of *Halomonas* sp. R5-57 were grown for two days at 20 °C to stationary phase. Growth media was in LB supplemented with 3.5 % NaCl. High molecular weight DNA was isolated using the GenElute Bacterial Genomic Kit (Sigma) following the manufacturer’s instructions for Gram negative strains. Briefly, cells were harvested by centrifugation from 1.5 ml culture, lysed in ‘Lysis solution T’ containing RNase A followed by treatment with Protease K. All subsequent steps involving binding to, and elution from spin columns were carried out according to the kit protocol, and the final genomic DNA sample was eluted in distilled water. Where mixing was required, gentle inversion of the sample was used in lieu of vortexing or pipetting to avoid shearing of the sample DNA. The DNA concentration was estimated by the absorbance at 260 nm, and purity was assessed by the ratio of absorbance at 260 to 280 nm measured on a Nanodrop spectrophotometer (Thermo scientific).

Genomic DNA was further prepared for Illumina sequencing by sonication using a Covaris sonicator down to ~700 bp, and the library was produced with Solid Phase Reversible Immobilization works technology (Beckman Coulter). The sample was then separated on a 2 % agarose gel (120V, 40 min) and DNA of 750-850 bp was retrieved. Afterwards PCR was performed to amplify the library.

**Genome sequencing and assembly**

Sequencing of *Halomonas* sp. R5-57 used a combination of Illumina and PacBio Single Molecule Real-Time (SMRT) technology. The finished sequence of *Halomonas* sp. R5-57 was completed in February 2015 and deposited in the European Nucleotide Archive [17] with the identifier LN813019 (GI:802125597).

Table 2 presents the project information and its association with MIGS version 2.0 compliance [18].

**Table 2 Project information**

| MIGS ID | Property | Term |
|---------|----------|------|
| MIGS 31 | Finishing quality | Finished |
| MIGS-28 | Libraries used | One Illumina Paired-End library, one 20 kb PacBio library |
| MIGS 29 | Sequencing platforms | Illumina HiSeq 2000, Pacific Biosciences PacBio RS II |
| MIGS 31.2 | Fold coverage | Illumina (512 ×), PacBio (16 ×) |
| MIGS 30 | Assemblers | Mira hybrid assembly |
| MIGS 32 | Gene calling method | Glimmer 3 |
| Locus Tag | HALO |
| Genbank ID | LN813019 |
| GenBank Date of Release | Mar. 31, 2015 |
| GOLD ID | Gs0114368 |
| BIOPROJECT | PRJEB8412 |
| MIGS 13 | Source Material Identifier | The skin of the red sea squirt Halocynthia papillosa collected from the Barents Sea |
| Project relevance | Biotechnological |
sequencing technology methods. Illumina sequencing (100 bp paired end) was done on a HiSeq2000 using TruSeq SBS v3 reagents (Illumina). This was followed by preparation of a PacBio library which was sequenced on the Pacific Biosciences PacBio RS II sequencer using P4-C2 chemistry [19]. The Illumina sequencing produced 26,184,828 raw reads (2,392,197 reads after removal of artifacts) giving an average genome coverage of 512 ×, and PacBio produced 10,611 raw reads (10,460 quality filtered) with a coverage of 16 ×. The reads were assembled using

**Fig. 3**

**a** Graphical representation of the 5.02 Mb chromosome of *Halomonas* sp. R5-57 indicating from innermost ring: distribution of the GC content (black), GC skew (purple/green), homology with self (solid purple), *H. elongata* DSM 2581 ASM19687v1 (green); *H. campaniensis* ASM69648v1 (pink), and *H. boliviensis* LC1 (blue). The outermost red blocks indicate areas where *Halomonas* sp. R5-57 has low homology with other species, and are annotated with possible genes of interest. The approximate position and locus tag of genes involved in ectoine biosynthesis are marked in blue, those producing PHA are in magenta.

**b** Comparison between *Halomonas* sp. R5-57 and *Halomonas* sp. TG39a. Low homology regions which have equivalent in part A are shown in red blocks with the position numbers of the *Halomonas* sp. R5-57 - those not identified in A are shown in green and also include possible genes of interest.

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MIRA hybrid assembly [20] which allowed mapping of the Illumina reads onto the PacBio scaffold for correction of indels, resulting in a single circular chromosome with no plasmids.

**Genome annotation**

Genes were identified using Glimmer 3 [21] and annotated using an in-house annotation pipeline where protein-coding sequences were searched against the COG database [22] and assigned with COG numbers, signal peptides were predicted using Phobius [23], and tRNA genes were identified using the tRNAscan-SE tool [24].

**Genome properties**

The genome comprises one circular chromosome of 5031571 bp which is graphically represented in Fig. 3a indicating the GC distribution (55.75 % overall) and GC skew. The properties and statistics of the genome are summarized in Tables 3 and 4. Four thousand six hundred seventy seven genes were predicted, 4599 of which are protein coding genes. Four thousand two hundred twenty five (91.87 %) of the protein coding genes were assigned to a putative function with the remaining genes annotated as hypothetical proteins.

**Insights from the genome sequence**

BRIG [25] was used to generate the comparison between the fully-genome sequenced species *H. elongata* DSM 2581 ASM19687v1 (4.06 Mb, 63.6 % G + C) and *H. campaniensis* ASM69648v1 (4.07 Mb, 52.6 % G + C), and the draft sequence of the type strain *H. boliviensis* LC1 (4.2 Mb, 54.7 % GC). The comparison was performed on the nucleotide sequences with a lower cut off identity threshold of 50 %. The genome comparison reveals several unique regions in the *Halomonas* sp. R5-57 genome. Most of these include mobile genetic elements, and some contain genes for membrane transporters, secretion proteins and restriction-modification systems (Fig. 3a). *Halomonas* sp. R5-57 has the highest overall similarity to the recently deposited High-Quality Draft sequence of *Halomonas* sp. TG39a (ASM74439v1; 4.9 Mb, 55.0 % G + C). A pairwise comparison using the nucleotide sequences of these two genomes and visualization in ACT [26] identified eight regions which differ between the two genomes: two of these appear to be translocations and correspond to parts of the *Halo- monas* sp. R5-57 which are not found in *H. elongata*, *H. campaniensis*, or *H. boliviensis*, five others are insertions which are unique to *Halomonas* sp. R5-57 and one is an insertion in *Halomonas* sp. TG39a Fig. 3b.

**Extended insights**

Species of *Halomonas*, like other halotolerant chemorganotrophic bacteria, produce compatible solutes to

### Table 3: Genome statistics

| Attribute                | Value   | % of Total |
|--------------------------|---------|------------|
| Genome size (bp)         | 5,031,571 | 100.00     |
| DNA coding (bp)          | 4,482,414 | 89.00      |
| DNA G + C (bp)           | 2,500,760 | 55.75      |
| DNA scaffolds            | 1       | 100.00     |
| Total genes              | 4,677   | 100.00     |
| Protein coding genes     | 4,599   | 98.33      |
| RNA genes                | 18      | 0.38       |
| Genes with function prediction | 3,356 | 71.75    |
| Genes assigned to COGs   | 4,225   | 91.87      |
| Genes with Pfam domains  | 4,406   | 94.20      |
| Genes with signal peptides | 1,605 | 37.99    |
| CRISPR repeats           | 64      | NA         |

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

### Table 4: Number of genes associated with general COG functional categories

| Code | Value | % of Total | Description                                      |
|------|-------|------------|-------------------------------------------------|
| J    | 210   | 4.6        | Translation, ribosomal structure and biogenesis  |
| A    | 1     | 0.0        | RNA processing and modification                  |
| K    | 397   | 8.6        | Transcription                                    |
| L    | 204   | 4.4        | Replication, recombination and repair            |
| B    | 7     | 0.2        | Chromatin structure and dynamics                 |
| D    | 36    | 0.8        | Cell cycle control, cell division, chromosome partitioning |
| V    | 64    | 1.4        | Defense mechanisms                               |
| T    | 262   | 5.7        | Signal transduction mechanisms                   |
| M    | 255   | 5.5        | Cell wall/membrane biogenesis                    |
| N    | 114   | 2.5        | Cell motility                                    |
| U    | 88    | 1.9        | Intracellular trafficking and secretion          |
| O    | 178   | 3.9        | Posttranslational modification, protein turnover, chaperones |
| C    | 300   | 6.5        | Energy production and conversion                 |
| G    | 340   | 7.4        | Carbohydrate transport and metabolism            |
| E    | 518   | 11.3       | Amino acid transport and metabolism              |
| F    | 93    | 2.0        | Nucleotide transport and metabolism              |
| H    | 198   | 4.3        | Coenzyme transport and metabolism                |
| I    | 180   | 3.9        | Lipid transport and metabolism                   |
| P    | 319   | 6.9        | Inorganic ion transport and metabolism           |
| Q    | 157   | 3.4        | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 626   | 13.6       | General function prediction only                 |
| S    | 379   | 8.2        | Function unknown                                 |
| -    | 374   | 8.1        | Not in COGs                                     |

The total is based on the total number of protein coding genes in the genome.
maintain the osmotic balance inside their cells. An example is ectoine which is produced by cultivation of strains *H. boliviensis* and *H. elongata* [27]. The genes of *Halomonas* sp. R5-57 involved in ectoine biosynthesis, hydroxylation and transportation, as well as for the production of PHAs are listed in Table 5 together with their predicted properties and locus tags. The approximate position of these genes is shown on the graphical representation of the *Halomonas* sp. R5-57 chromosome (Fig. 3a). High homology is found between the two EctD protein products of *Halomonas* sp. R5-57 and *H. boliviensis* (89 % and 99 %) as well as their EctA, EctB, and Ect C sequences (98, 98 and 85 %). Homology is slightly lower between *Halomonas* sp. R5-57 and *H. elongata*: EctDs (69 % and 73 %) EctA (85 %), EctB (86 %), and Ect C (81 %).

PHAs are cellular energy-storage molecules that can serve as precursors for bioplastic production by humans, [12, 28]. *Halomonas* sp. R5-57 carries three genes annotated as polyhydroxyalkanoate syntheses (PHA Cs); the enzymes responsible for carrying out the final polymerization step in PHA biosynthesis [28]. The product of *phaC* HALO1802 has high homology with PHA C1sequences of *H. boliviensis* (91 %) and *H. campaniensis* (86 %) and with enzymes from *Halomonas* spp.O-1 (86 %) and *H. elongata* (77 %) which have recently been heterologically produced and characterized [29]. The putative PHA C (HALO2716) of *Halomonas* sp. R5-57 differs from the PHA C1 sequences, but has 75 % homology with another PHA C from *H. boliviensis*. A third possible PHA C comprising loci HALO3139 and HALO3140 contains a frameshift generating a stop codon after 67 amino acids, and is found within the phage-containing poorly-conserved 3367–3491 kbp region of the *Halomonas* sp. R5-57 genome (Fig. 3a). The *phaC* genes of *Halomonas* sp. R5-57 have been cloned, and their recombinant expression and structural elucidation is part of ongoing studies by our group to more fully understand the biochemical properties and catalytic mechanism of these enzymes.

Given its ability to tolerate salt concentrations up to 12 %, extracellular enzymes from *Halomonas* sp. R5-57 are expected to be functional under moderate-to-high salt conditions and thus could be employed in high-salinity reaction conditions. Functional screening of *Halomonas* sp. R5-57 using the API® system and plate-based assays revealed several secreted enzyme activities that could be of interest in industrial and biotechnological settings. Subsequent to genome sequencing, the genes annotated with enzyme classes that could impart these functions were identified together with putative signal peptides for secretion (Table 6).

A further possible application for *Halomonas* sp. R5-57 would be manipulation of its cellular machinery for use as a protein-expression host. The low-temperature and high-salinity growth optima could be potentially advantageous for recombinant production of psychrophilic or halophilic enzymes, which can suffer from poor solubility in commonly-used E. coli-based expression systems. Additionally, as osmolyte compounds are known to be potent protein stabilizers [30], their induction simultaneously with intracellular heterologous protein expression in *Halomonas* could present a further strategy to improve solubility of ‘difficult’ recombinant protein targets. The in-depth sequence information of halophilic bacterial strains, such as we have provided in this project will be key to engineering of such organisms in realization of this goal.

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**Table 5** Genes from *Halomonas* sp. R5-57 predicted to be involved in production of ectoine and PHAs

| Solute | Gene product | Function | Locus tag | MW (kDa) | pI |
|--------|--------------|----------|-----------|----------|----|
| Ectoine | EctD | Ectoine hydroxylase | HALO0980 | 36.7 | 5.5 |
| | 5-carboxymethyl-2-hydroxymuconate delta-isomerase | HALO0981 | 24.1 | 4.8 |
| | EctA | L-2,4-diaminobutyric acid acetyltransferase | HALO2492 | 21.1 | 5.0 |
| | EctB | Diaminobutyrate-2-oxoglutarate transaminase | HALO2491 | 46.1 | 5.8 |
| | EctC | Ectoine synthase | HALO2490 | 14.7 | 5.0 |
| PHA | PHA B | acetoacetyl-CoA reductase | HALO4132 | 26.8 | 5.62 |
| | PHA A | Acetyl-CoA acetyltransferase | HALO1802 | 71.8 | 4.9 |
| | PHA A | Acetyl-CoA acetyltransferase | HALO1910 | 41.0 | 6.0 |
| | PHA A | Acetyl-CoA acetyltransferase | HALO2333 | 41.8 | 5.5 |
| | PHA A | Acetyl-CoA acetyltransferase | HALO4196 | 40.5 | 5.6 |
| | PHAC | PHB synthase | HALO2716 | 66.7 | 5.3 |
| | PHAC | PHB synthase truncated | HALO3139 | na | na |
| | PHAC | PHB synthase | HALO3140 | na | na |
Table 6 Enzyme activities detected by functional screening

| Putative function (E. C. number) | Genes | Activity | Total | Signal peptides |
|---------------------------------|-------|----------|-------|----------------|
| Triglycerol lipase (3.1.1.3)    | 4     | Lipase   | 4     |                |
| Hydrolases acting on peptide bonds (protease, 3.4.-) | 43 (20) | Gelatinase | 10 |                |
| Glycosidases hydrolysing O- and O-glycosyl compounds (3.2.1.-) | 14 | Chitinase | 2 |                |
| Exodeoxyribonuclease (3.1.11.-) | 6 | DNAse | | |
| Endodeoxyribonuclease (3.1.21.-) | 1 | DNAse | | |
| Hydrolases acting on C-N bonds in linear amidines (3.5.3-) | 7 | Arginine dihydrolase | | |
| Nitrate reductases (1.7.99.4)    | 1     | Nitrate reduction | | |

Conclusions

*Halomonas* sp. R5-57 has several phenotypic and genetic features, which may impart useful properties in biotechnological applications. The complete genome sequence of *Halomonas* sp. R5-57 presented here will help utilization the biotechnological potential of this organism; either by whole-cell cultivation for production of high-value products such as ectoine and PHAs, or as a source of gene-mining for individual enzymes.

Additional file

Additional file 1: Figure S1. Temperature and salinity optima of *Halomonas* sp. R5-57 grown in LB media. The growth rate represents the increase in absorbance at 600 nm during the exponential growth phase of cultures. (PNG 57 kb)

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Authors’ contributions

AW selected *Halomonas* sp. RS-57 for genome sequencing, BA and CK conducted salinity and temperature-dependent growth measurements. AW and CD conducted metabolic and functional screening. EH carried out genome assembly, annotation and other bioinformatic analyses. All authors approved the manuscript and its submission.

Competing interests

The authors declare that they have no competing interests.

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