# Whole-Genome Analysis of *Halomonas* sp. Soap Lake #7 Reveals It Possesses Putative Mrp Antiporter Operon Groups 1 and 2

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## Abstract

The genus *Halomonas* possesses bacteria that are halophilic or halotolerant and exhibit a wide range of pH tolerance. The genome of *Halomonas* sp. Soap Lake #7 was sequenced to provide a better understanding of the mechanisms for salt and pH tolerance in this genus. The bacterium's genome was found to possess two complete multiple resistance and pH antiporter systems, Group 1 and Group 2. This is the first report of both multiple resistance and pH antiporter Groups 1 and 2 in the genome of a haloalkaliphilic bacterium.

**Key words:** Mrp antiporter operon system, *Halomonas*, pH tolerance, salt tolerance, extremophiles.

## Introduction

The genus *Halomonas* belongs to the family of *Halomonadacea* with the type strain *Halomonas elongate* first described by Vreeland et al. (1980). The members of *Halomonas* are halotolerant or strictly halophilic and most are capable of tolerating a wide range of pH values including *H. campisalis* (pH range 6–12), *H. alkaliantarctica* (pH range 7.4–9.6), and *H. olivaria* (pH range 5–11) (Mormile et al. 1999; Poli et al. 2007; Amouric et al. 2014). These organisms must possess mechanisms to tolerate changing osmotic pressures and maintain pH homeostasis as salt and pH conditions vary in their environment. Multiple resistance and pH (Mrp) antiporter systems are reported to provide these functions (Ito et al. 2017).

Mrp antiporter systems are multicistronic operon systems that catalyze the efflux of monovalent cations ($Na^+$, $K^+$, and $Li^+$) and facilitate the influx of protons (Fang et al. 2018). These systems are found in a wide phylogenetic range of organisms (Swartz et al. 2005). There are two known Mrp antiporter systems; Group 1 and Group 2. Group 1 possesses seven genes (*mrpABCDEFG*), whereas Group 2 possesses six genes with the first gene *mrpA*′ being comprised both the *mrpA* and *mrpB* domains. All genes have to be present in each of these operons to be functional (Swartz et al. 2005).

## Materials and Methods

### Isolation of *Halomonas* sp. Soap Lake #7

The bacterium used in this study was isolated from sediment samples obtained from Soap Lake, Washington, a meromictic haloalkaline soda lake. The pH of the lake is 9.8 and the anaerobic sediments of the lake can contain upward of 140 g/l NaCl (Mormile et al. 1999). Collected samples were pasteurized for 90 s in a boiling water bath to select for *Bacillus*-like bacteria and used to inoculate enrichment cultures. The medium used for enrichment of haloalkaliphilic bacteria contained 8 g/l Difco nutrient broth (Becton Dickinson, New Jersey) and 120 g/l NaCl. The pH of the

medium was adjusted to 9 with 1 M NaOH. After autoclaving, the medium was supplemented with filter-sterilized glucose as a carbon source to provide a final concentration of 1% (v/v). Cultures were incubated in a shaking incubator at 30 °C until turbid. Agar-solidified medium was inoculated and individual colonies were subsequently streaked onto fresh medium for strain isolation. Salt nitrate medium was used to determine if $Na^+$ is required for growth (Mormile et al. 1999). This medium contains in grams per liter: $KH_2PO_4$, 0.5; $NH_4Cl$, 1.0; yeast extract, 0.25; $NaNO_3$, 3; NaCl, 75; and adjusted to pH 9 using 1 M NaOH. To determine specific ionic requirements, all sodium was removed from the base medium and substituted with either $K^+$, $Ca^{2+}$, or $Mg^{2+}$ salts.

### DNA Extraction and Genome Sequencing

The isolates were cultured in fresh enrichment medium and incubated 72 h at 30 °C. Genomic DNA of the culture was isolated by using the QIAamp DNA Mini kit protocol and eluted with 10 mM Tris HCl buffer solution. The sequencing libraries were prepared according to the Nextera XT DNA Library Prep Kit protocol and sequenced on the Illumina MiSeq platform using a MiSeq V3 600-cycle reagent kit with 300-bp paired-end reads.

### Genome Assembly

A clone of the isolate strain obtained under the same conditions and time *Halomonas* sp. Soap Lake #6 was cultured and sequenced on the Pacific Biosciences (PacBio) RS II Sequencer as previously described (Yao et al. 2017). A complete genome sequence was produced by de novo assembly with the PacBio Hierarchical Genome Assembly Process (HGAP3.0) program by using continuous-long-reads from the four SMRT cells. The complete genome sequence of *Halomonas* sp. Soap Lake #6 (GenBank accession number CP020469) was used for referenced-based assembly of the *Halomonas* sp. Soap Lake #7.

*Halomonas* sp. Soap Lake #7 sequence reads were aligned to the Soap Lake #6 reference chromosome by using the Bowtie2 software (V2.1.0, Langmead and Salzberg 2012). The alignment map was then used to generate a consensus sequence by using SAMtools (V0.1.9, Li et al. 2009). The consensus genome was annotated by using the NCBI's Prokaryotic Genomes Automatic Annotation Pipeline (Klimke et al. 2009).

### Genomic Analysis

Information on the presence of possible antiporter systems and their locations were obtained by using the Integrated Microbial Genomes system (Markowitz et al. 2012). Genomic similarity of *Halomonas* sp. Soap Lake #7 and its Mrp operons were determined by using a Microbial Nucleotide BLAST (Altschul et al. 1990).

**Table 1**
Genomic Statistics of *Halomonas* sp. Soap Lake #7

| | Number or % of Total |
|---|---|
| Total number of bases | 4,803,686 |
| Number of DNA coding bases | 4,317,101 |
| G + C content (%) | 52.71 |
| Total number of protein-coding genes | 4,279 |
| 5S rRNA genes | 6 |
| 16S rRNA genes | 6 |
| 23S rRNA genes | 6 |
| tRNA genes | 60 |
| Antiporter genes | 26 |
| Genome coverage | 60× |
| GenBank accession number | CP019915 |

## Results and Discussion

### pH and Salt Tolerance

Growth was observed at pH 7–11 with optimal pH occurring at pH 9 for *Halomonas* sp. Soap Lake #7. The organism can grow on nutrient broth medium without $Na^+$ addition and with NaCl supplementation up to 250 g/l. Optimum growth occurred at 100 g/l. However, no growth was seen when $Na^+$ was removed from defined salt nitrate medium indicating that $Na^+$ is required for growth.

### Putative Presence of Both Group 1 and 2 Mrp Systems

*Halomonas* sp. Soap Lake #7 consensus sequence consisted of a single chromosome with a length of 4,803,686 bp, a G/C content of 52.71% and 4,279 coding genes. The complete assembly can be found in GenBank with the accession number CP019915. One of the goals of our research is to better understand the mechanisms haloalkaliphilic bacteria possess to tolerate wide ranges of pH and salinity. *Halomonas* sp. Soap Lake #7 appears to possess 26 antiporter transport genes in its genome (table 1). It has been suggested by Krulwich et al. (2009) that the number of antiporter genes that occur in an organism is related to the diversity of environmental challenges it is exposed to. Among the putatively identified genes, both Group 1 and 2 Mrp genes that confer tolerance to both alkaline and saline conditions were present. Group 1 possessing *mrpABCDEFG* is located on the minus strand of the chromosome from coordinates 3058386 to 3055269, whereas Group 2 possessing *mrpA′CDEFG* is present on the plus strand from coordinates 3061111 to 3067024. There is a gap of 373 nucleotide units between these operons. Their close proximity and inversion to one another suggests that their regulation is coordinated.

### Genomic Similarity of *Halomonas* sp. Soap Lake #7 and Its Mrp Operons to Other Reported *Halomonas* Species

A number of *Halomonas* species possessed sequences with alignment sequences >90% similarity to *Halomonas* sp. Soap

Lake #7 genome. However, query coverages were 46% or lower. Specific examples include *Halomonas campaniensis* strain LS21 (GenBank accession #NZ_CP007757.1) that possesses a percentage identity of 92.7% with a 46% query coverage and *Halomonas boliviensis* LC1 Scaffold 1 (GenBank accession #NZ_JH393257.1) possessing percentage identification of 92.6% with a query coverage of 19%. Other *Halomonas* species had query coverage that dropped to between 10% and 0% and percentage identities between 97.7% (0% query coverage) and 75.5% (0% query coverage). No *Halomonas* species were found to possess sequence similarities with the complete Group 1 and Group 2 Mrp operons of *Halomonas* sp. Soap Lake #7. Taken individually though, *Halomonas* sp. GFAJ-1 (GenBank accession # CP016490.1) did possess similarities to Group 1 genes Mrp A, Mrp B, Mrp C, and Mrp D (82.7%, 89.1%, 85.1%, and 83.2%, respectively), and Group 2 Mrp A′ and Mrp G (79.4% and 83.3%, respectively).

Previously, Meng et al. (2014) demonstrated the presence of a novel Group 1 Mrp operon capable of functioning as a pH-dependent antiporter system with similar $Na^+$, $Li^+$, and $K^+$ affinities in *Halomonas zhaodongensis*. By using transcriptional analysis and mutant studies, Cheng et al. (2016) also found a pH-dependent antiporter Group 1 Mrp operon in *Halomonas* sp. Y2. Likewise, Zhai et al. (2018) found similar activity in *Halomonas alkalicola*. As far as we are aware, this is the first report of both Group 1 and 2 Mrp operons present in haloalkaliphilic bacteria such as *Halomonas*.

## Conclusion

The genome sequence of *Halomonas* sp. Soap Lake #7 indicates that it possesses multiple antiporter genes including both Groups 1 and 2 of the Mrp operons. Group 1 has previously been identified and studied in three different species of *Halomonas*. The presence of these genes is suggestive of mechanisms that extremophiles can use to tolerate high salt and alkaline conditions. Only one bacterium, *Sinorhizobium meliloti* has been previously reported to possess both Group 1 and 2 Mrp operons (Yamaguchi et al. 2009). *Sinorhizobium meliloti* is a nitrogen-fixing bacterium that requires Pha1 (Mrp1) for $K^+$ efflux and to invade plant nodules (Putnoky et al. 1998). However, its role for Mrp 2 has not been reported yet. The role of the Group 2 Mrp operon was discovered in a halophilic *Dietzia* sp. DQ12-45-1b to be critical in its response to high alkaline shock (Fang et al. 2018). The presence of both Mrp operon systems in *Halomonas* sp. Soap Lake #7 provides a unique opportunity to study the function of these systems and to better understand how haloalkaliphilic bacteria can thrive under broad pH and saline conditions.

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## Author Contributions

M.R.M. and J.M. designed the study. T.E. isolated and characterized isolates Soap Lake #6 and #7. M.H. performed the PacBio sequencing. Z.G. performed the Illumina sequencing and assembly. M.R.M., T.E., R.F., and J.H. analyzed and interpreted the data. M.R.M. drafted the manuscript. All authors read, edited, and approved the final manuscript.

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