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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 Halomonadaceae 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 (Itto 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 mrpA0 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: KH₂PO₄, 0.5; NH₄Cl, 1.0; yeast extract, 0.25; NaNO₃, 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⁺, Ca²⁺, or Mg²⁺ 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).

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 Kruilwich 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 mrpABCDEF is located on the minus strand of the chromosome from coordinates 3058386 to 3055269, whereas Group 2 possessing mrpA’C’D’E’F’ 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.

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 |
| 16S rRNA genes | 6 |
| 16S rRNA genes | 6 |
| 23S rRNA genes | 6 |
| tRNA genes | 60 |
| Antipoter genes | 26 |
| Genome coverage | 60× |
| GenBank accession number | CP019915 |

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 campensis 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\(^{+}\) efflux and to invade plant nodules (Putnoky et al. 1998). However, its role for Mrp 2 has not been reported previously. Sinorhizobium meliloti, a denitrifying, exopolysaccharide-producing bacterium. Syst Appl Microbiol. 30(1):31–38.

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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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