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Characterization of Ten Microbial Isolates from Two Serpentinite Seamounts, Asùt Tesoru and Fantangisña, in the Mariana Forearc

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Biology

by

Brontë Shelton

Committee in charge:

    Professor Douglas H. Bartlett, Chair
    Professor Eric Allen
    Professor Jeff Bowman

2020

The Thesis of Brontë Shelton is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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2020

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ABSTRACT OF THE THESIS

Characterization of Ten Microbial Isolates from Two Serpentinite Seamounts, Asùt Tesoru and

Fantangisña, in the Mariana Forearc

by

Brontë Shelton

Master of Science in Marine Biology

University of California San Diego, 2019

Douglas H. Bartlett, Chair

The Mariana Forearc is home to the only known active serpentinite seamounts on Earth.

Serpentinization, a reaction that occurs when ultramafic rock is exposed to water, fuels these

unique environments. These seamounts are home to microbial communities that have barely

begun to be explored; only one species of bacteria had been isolated and described prior to this

research. During International Ocean Discovery Program Expedition 366, sediment samples

were obtained from three seamounts. In this research, I characterized ten strains of bacteria

isolated at atmospheric pressure from samples obtained from two of the seamounts, Asùt Tesoru

and Fantangisña. All of the isolates are closely related to previously cultured microbes and represent three genera: *Halomonas, Demequina,* and *Marinobacter.* These ten isolates were examined for pH tolerance, pressure tolerance, salinity requirement and tolerance, and temperature tolerance. The majority of these isolates were pressure-sensitive and alkaliphilic. The only previously characterized bacterium isolated from the seamounts, *Marinobacter alkaliphilus* str ODP1200D-1.5, was obtained from the Japan Collection of Microorganisms, tested alongside the isolates, and sent for genome sequencing along with three of the isolates. Genomic analyses revealed several adaptations and metabolic capabilities that could contribute to survival in the seamounts, including Na^+/H^+ antiporters and acetate metabolism. The results of this research indicate that the characterized isolates could be active *in situ*, and therefore likely represent a portion of the active community at the seamounts. This thesis contributes to the knowledge of the microbial communities and adaptations required for life at serpentinite seamounts.

INTRODUCTION

The deep sea is home to an incredible diversity of life in many extreme habitats, where organisms must endure a myriad of challenges such as high hydrostatic pressure, low light availability, and temperature ranges from -2°C in the Antarctic to 400°C at hydrothermal vents (Gordon 2001, Zierenberg et al. 2000). One such place of interest is the Mariana Trench, a subduction zone where the Pacific plate is overridden by the Philippine plate, creating an array of extreme conditions. Along the length of the Mariana Forearc, the area between the trench axis and the volcanic arc, the subduction of the Pacific plate has given rise to a series of seamounts extending up to 120 kilometers (km) away from the trench axis, with summits at depths down to 3.4km below sea level (Fig. 3) (Fryer et al. 2018b, Mottl et al. 2003). These seamounts are serpentinite mud volcanoes, and are the only known active site of serpentinite eruption in the world (Fryer 1996). Serpentinizing systems have been implicated as the site of the origin of life on Earth and studying these systems could provide novel insights into these origins as well as an invaluable analogue to such systems on other planets (Hastie et al. 2016, Wilde et al. 2001). Understanding the microbial communities at these sites will provide a window into places such as Enceladus (one of Saturn's moons) and Mars, where serpentinization is believed to occur, furthering the search for life elsewhere in the solar system (Glein et al. 2015, McCollom and Seewald 2013, Schulte et al. 2006).

Serpentinization is a chemical process that occurs when ultramafic rock, which is high in Iron (Fe) and Magnesium (Mg), reacts with water, such as in serpentinizing hydrothermal systems or at convergent margins like the Mariana Trench. The hydration of this mantle material results in the production of a group of serpentine minerals, the generalized chemical composition of which is $[(Mg,Fe)_3Si_2O_5(OH)_4]$, as they are formed through the hydration of minerals such as olivine $[(Mg,Fe)_2SiO_4]$ and enstatite $[MgSiO_3]$ in the mantle of the overriding plate (Plümper et

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al. 2017, Shervais et al. 2005). The resulting reactions, such as [(Mg,Fe)$_2$SiO$_4$ + H$_2$O →

[(Mg,Fe)$_3$Si$_2$O$_5$(OH)$_4$] + H$_2$], cause a decrease in the density of the material and, when iron is

present in the parent mineral, produce a significant amount of hydrogen (Preiner et al. 2018,

Shervais et al. 2005). In the case of plate subduction, water from the subducting plate is able to

react with the mantle of the overriding plate. The decrease in density allows the metamorphosed

rock to rise to the seafloor through fractures in the overriding plate, resulting in serpentinite

volcanism (Fryer et al. 2020). The resulting habitats can have extremely high pH and high

concentrations of methane, hydrogen, and, importantly, short chain organic acids such as

formate, acetate, and propionate that could provide a high energy substrate for microbial

communities (Eickenbusch et al. 2019, Schrenk et al. 2013). The release of large amounts of H$_2$

during the hydration of olivine has been shown to be a source of energy for hydrogen oxidizing

bacteria (Plümper et al. 2017).

The seafloor of the Pacific Plate is home to many ancient seamounts and reefs that, while

no longer active, create significant topographical relief (Haggerty and Fisher 1992). When these

features come into contact with the overriding plate, they must be accommodated in order for

subduction to continue. While it was previously thought that these features were crushed and

distorted by the process of subduction, more recent observations in the Mariana Trench have

shown that even large formations can be subducted in a relatively intact state (Fryer et al. 2020,

Watts et al. 2010). This means that not only does the overriding plate experience considerable

deformation and faulting when these features are subducted, but also that the features themselves

experience a range of physical conditions due to their relief once they enter the subduction

channel (Fryer et al. 2020). The faults created by these structures are key to the eventual

formation of serpentinite seamounts in the forearc, as they are the avenues that allow the newly

buoyant serpentinite muds to rise to the surface. In terms of the gradient of conditions that these structures can experience, the bottom of a subducted seamount, for example, is more rapidly subject to significantly higher pressures and temperatures than the summit. Analysis of recovered seamount and reef fragments has shown that these summits do not necessarily enter depths or temperatures that would be lethal to microbial life, with measurements as low as 300 megapascals (MPa) and 80°C (Fryer et al. 2020). Bacteria have been shown to be able to recover from pressures above 700 MPa, and temperatures as high as 130°C (Cheftel 1995, Kashefi 2003, O'Reilly et al. 2000). It is therefore possible that microbial communities living on the seafloor of the Pacific Plate could be subducted on a high relief structure, travel through the mantle of the overriding plate, and rise to the seafloor entrained in the serpentinite mud and fluids to find a new habitat at the seafloor of the Philippine Plate, where the temperatures are low and the pressure at the summits of the seamounts is not above 50MPa (Fryer et al. 2020).

Serpentinite systems have existed in the Earth's oceans for as many as 4 billion years and are a candidate for the locus of life's origin on Earth (Fryer et al. 2020, Hastie et al. 2016, Wilde et al. 2001). While serpentinizing hydrothermal vents have garnered much attention as the potential site of the first cellular life, serpentinite seamounts share all of the essential characteristics as well as a few key advantages. A universally agreed upon tenant for the origin of life is the existence of stable, liquid water and both hydrothermal vents and serpentinite seamounts clearly fulfill this condition (Darwin 1871, Deamer et al. 2006). The availability of raw biotic materials, which include biotic elements, minerals, and the simple biotic molecules such as H_2, CH_4, and NH_4, is also not disputed at either site (Barge et al. 2017, Deamer et al. 2006). An important element in the equation is the concentration of these raw biotic materials and, while mechanisms have been proposed for the concentration of prebiotic molecules in the

crevices of hydrothermal vents, the episodic eruption of the serpentinite seamounts offers a clear method of concentration with periodic inputs of fresh mud and fluids with ample time in between eruptions for the settling of prebiotic molecules (Fryer et al. 2020, Sleep et al. 2011). A source of energy for reactions to progress is also a crucial ingredient, and the vents and the seamounts share a mechanism for this as well. Serpentinization in both environments provides a mechanism for the generation of a chemical potential gradient in the form of a proton motive force, fueled by the difference in pH between the serpentinizing fluids and the surrounding seawater (Russel et al. 2010, Sleep et al. 2011). Earth's early oceans are predicted to have been significantly more acidic than their current pH of about 8.1, with predictions for the late Hadean and early Archean oceans ranging from a pH of 5.1 to a pH of 7 due to the increased concentration of CO_2 in the atmosphere (Halevy and Bachan 2017, Sleep et al. 2011). The fluids escaping both the vents and the seamounts are generally between a measured pH of 8 and 12; this sharp gradient would have facilitated a proton motive force as well as a significant redox potential (Fryer et al. 2020, Lane et al. 2010, Russel et al. 2010, Sleep et al. 2011).

The admitted drawbacks to the theory that life originated at hydrothermal vents include the high temperatures, limited spatial range, and ephemeral nature of these systems (Fryer et al. 2020, Sleep et al. 2011). The temperatures at hydrothermal vents have been proven to be too high for the formation of stable biomolecules and, while some argue that the cooler surrounding environment would have been sufficient, serpentinite seamounts do not suffer this disadvantage as they are not located at spreading centers (Fryer et al. 2020, Peresypkin et al. 1999). The short-lived nature of hydrothermal vents would have presented another impediment to the formation of the first cells, with longevity estimates up to tens of thousands of years for the systems and less than 1,000 years for individual vents, while the seamounts can remain stable for millions of years

(Brazelton et al. 2010, Fryer and Salisbury 2006, Ludwig et al. 2005). The constraints on dispersal within and between hydrothermal vent ranges is a subject of much study, and the current consensus is that there is very little connectivity, at least in the modern oceans (Bada 2002, Tyler and Young 2003). Serpentinite seamounts, conversely, are further benefitted by their location on subduction zones, where they occur in high densities and which span much larger ranges than hydrothermal systems and would more easily facilitate dispersal of the first cellular life on Earth throughout the oceans (Fryer et al. 2012). Although the Mariana Trench is the only known active site of subduction zone serpentinization today, there is evidence of such systems in former convergent margins worldwide from the California coast to the Isua Formation in Greenland, dating back to the Eoarchean and showing how widespread these habitats could have been (Fryer et al. 2020, Hess 1955, Pons et al. 2011).

The alkalinity of serpentinizing environments necessarily indicates that alkaliphilic organisms, who grow best at a pH of 9 or greater, and alkalitolerant organisms, who grow best at more neutral pH but can tolerate an elevated pH, would dominate any existing microbial communities (Fryer et al. 2020, Horikoshi 2004). Whether the microbes made their way to the seamounts via subduction and eruption, or through less extreme means such as recolonizing the fluids as they rose through the plate or settling out of the water column onto the seafloor, they would have to be able to adapt in order to survive. While alkaliphiles have played a role in human processes, such as the production of indigo in Japan, for centuries, the study of alkaliphiles and their adaptations did not begin until the mid-1900s and did not gain much traction until later in the century. Dr. Koki Horikoshi identified the first alkaline enzymes in 1971, and thereafter coined the term "alkalophiles" (Horikoshi 1971, Horikoshi and Akiba 1985). Since then, alkaliphiles have been identified in every domain of life and their adaptations

have led to unprecedented advances in many industrial fields (Horikoshi 1997). Known adaptations to alkaline environments include regulation of intracellular pH, specialized ATP synthesis, and a suite of specialized enzymes.

The ability to regulate intracellular pH is a trait shared by all known alkaliphiles (Horikoshi 2004, Krulwich et al. 2007). By maintaining an intracellular pH more than 2 pH units lower than the extracellular environment, they are able to thrive in much more alkaline conditions than neutralophilic microbes. A core mechanism of this homeostasis is a sodium-proton (Na^+/H^+) antiporter, which, while present in neutralophiles, is more common, ion-specific, and robust in alkaliphiles (Matsuno et al. 2018, Padan et al. 2005). These antiporters take up H^+ ions and expel Na^+ ions from the cytoplasm, with a greater number of H^+ ions taken up than Na^+ ions expelled each time (Krulwich et al. 2011). This has a two-fold advantage for the cell, as a net positive charge is generated at each exchange, maintaining a more acidic interior, and the cytotoxic effects of excess Na^+ are avoided since it is being selectively expelled (Krulwich et al. 2007). An additional benefit of this mechanism is that the removal of Na^+ ions from the cytosol creates a chemical gradient that alkaliphiles take advantage of in the form of the sodium motive force, which facilitates processes such as the co-transport of nutrients (Krulwich et al. 2007). Neutralophiles often employ a proton motive force to move important solutes into the cell, but in an alkaline environment there is not an excess of external H^+ ions; alkaliphiles must therefore use another strategy to bring substrates into the cell. By employing Na^+ ions as co-transporters, they are able to obtain key solutes as well as continuously replenish their Na^+ supply, which can then be expelled in exchange for H^+ ions to maintain pH homeostasis, as described above (Horikoshi 1999). These Na^+/solute symporters do not appear to employ any other ions to facilitate co-transport, and can even be inhibited by higher extracellular

concentrations of H^+; this also points to the reason why most alkaliphiles require sodium for growth (Guffanti and Krulwich 1992, Horikoshi 1999).

Since alkaliphiles maintain a more acidic interior than their alkaline environment, mechanisms to generate chemiosmotic energy at neutral conditions are not adequate to drive many crucial cellular processes. Non-fermentative organisms use F_1F_0-ATP synthase to produce ATP, and synthesis of ATP in this manner is dependent on the generation of a sufficient proton motive force, which relies on the presence of sufficient proton and charge gradients across the inner membrane (Fujisawa et al. 2010, Matsuno et al. 2018). By inverting the bulk proton gradient between the extracellular and intracellular compartments, alkaliphiles are only able to produce a very small proton motive force (Krulwich et al. 2007). There are several proposed mechanisms by which alkaliphiles overcome this barrier to ATP production, although it is important to note that thus far the majority of studies have been conducted on *Bacillus* species. Variations in the amino acid residues of the ATP synthase of *Bacillus* species have been shown to help trap and retain protons and facilitate their passage to the synthase rotor (Fujisawa et al. 2010). The components of the electron transport chain are also more abundant in alkaliphilic *Bacillus* species than in neutralophilic microbes (Hicks and Krulwich 1995). Another proposed adaptation is that the charged cell wall of alkaliphiles might help trap H^+ ions and prevent them from quickly diffusing away from the ATP synthase (Preiss et al. 2015). Inactivation of any of these crucial mechanisms has been shown to disable or severely impair growth in alkaliphilic media (Krulwich et al. 2001, Preiss et al. 2015).

The potential for the existence of extremophilic microorganisms, alkaliphilic or otherwise, at the serpentinite seamounts makes these environments a subject of much interest. The seamounts expel fluids periodically for up to millions of years, producing stable

environments with a measured pH above 12.5, and yet there is evidence that communities are thriving on these serpentinite mud volcanoes in such extreme conditions (Fryer and Salisbury 2006). Prior to this research, only two seamounts in the Mariana Trench, Conical Seamount and South Chamorro Seamount, had been examined for microbial communities and published data on the subject exists only for South Chamorro Seamount (Fryer et al. 1990, Mottl et al. 2003). A community dominated by archaea in the top 20m of sediment was discovered in 2001, and further studies resulted in the isolation of a novel species of bacteria, *Marinobacter alkaliphilus* sp. nov., from the top 1.5m of sediment (Mottl et al. 2003, Takei et al. 2005). Multicellular organisms including tube worms and mussels, likely utilizing methane and sulfur, have been collected from the surface of several seamounts in the past (Fryer et al. 1990, Fryer and Mottl 1997, Mottl et al. 2003). However, microbial biomass has been observed to be low unless the communities are near the surface, and diversity of the microbial and macrofaunal communities is low in general, due to the extreme nature of the habitats (Schrenk et al. 2013). The microbes in this study were obtained from core samples taken from two of the three serpentinite seamounts sampled along the Mariana Forearc during International Ocean Discovery Program (IODP) Expedition 366: Asùt Tesoru and Fantangisña (Fig. 1). The third seamount, Yinazao, is not represented in this analysis because incubation of the sediment samples did not yield any colonies at any test condition. Each mud volcano can be the result of multiple fissures transporting material from any number of depths (Fryer et al. 2020). The combination of source materials and fluids all contribute to the resulting ecosystem. Thusly, each seamount is unique and provides its own contribution to understanding the processes of subduction and the adaptations of resident microbial communities.
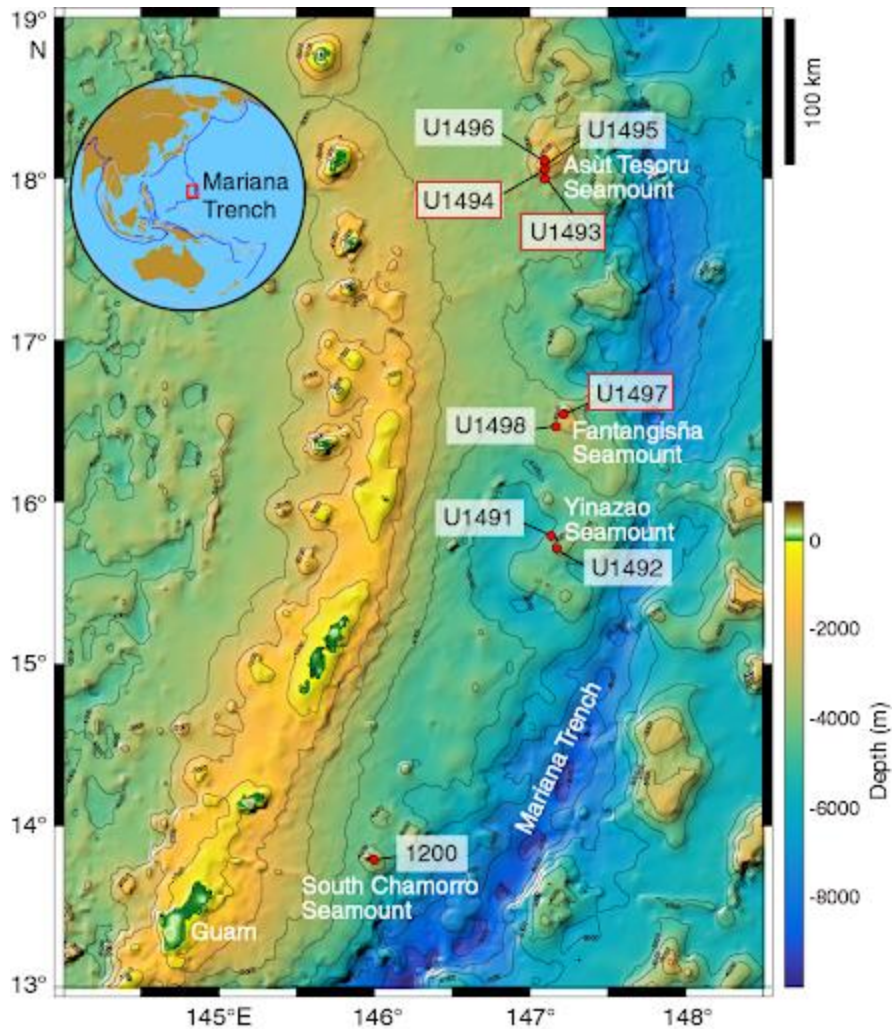
Figure 1. Diagram of the Mariana Trench and Relevant Seamounts. Modified from Fisher et al. 2020. Red boxes indicate samples that are represented in this study

The sediment sample from Fantangisña, site U1497B, was obtained from a drill core taken at the summit of the seamount, 2,018 meters below sea level (mbsl), that reached 34.2 meters below seafloor (mbsf). The seamount is located 62km from the trench and 14km above the subducting Pacific Plate (Fryer et al. 2020). The interstitial water at this site had the highest concentration of calcium of all the sample sites, and the pH was measured at 7.87. Incubation at atmospheric pressure of this sediment yielded 31 isolates representing two genera and three species. Two of these isolates are included in this analysis.

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The seamount Asùt Tesoru is of particular interest, as it has been proven to harbor archaeal and multicellular life at its surface (Curtis et al. 2013). This seamount is located 72km behind the trench and 18km above the subducting Pacific Plate (Fryer et al. 2018b) Two sediment cores from this seamount are represented in this study. The first was obtained at site U1493B from the flank of the seamount at 3,359mbsl and reached 32.6mbsf, with the pH of the interstitial water measured at 8.35. Plating of this sediment yielded 38 isolates representing four genera and ten species, six of these isolates are included in this analysis. The second was obtained at site U1494A from the flank of the seamount at 2,220mbsl and reached 39mbsf, two sediment samples from different sections of this core are represented here; one section was not subjected to chemistry measurements at the time of collection but a section 1.4m away had a measured pH of 9.01; the pH of the interstitial water of the second section was measured at 8.7. These two sediment samples collectively yielded 43 isolates representing two genera and three species, two of these isolates are included in this analysis. Asùt Tesoru has also shown a variance in chemical output from the other seamounts and has an extreme pH at the summit measured as high as 12.5 (Fryer et al. 2018b, Hulme et al. 2010). It has been shown that seamounts that are further from the Mariana Trench have decreasing concentrations of calcium and strontium, further separating the environment of Asùt Tesoru from other seamounts (Hulme et al. 2010).

The focus of this research is the characterization of cultured microbes isolated from the sediment samples at atmospheric pressure (0.1 MPa). Determining what kinds of microbes exist in these samples is the key first step in understanding these habitats. The identities and metabolic capabilities of these isolates will contribute to the characterization of the seamounts, revealing what makes the seamounts habitable for these organisms. A subset of ten representative isolates were chosen for a number of characterization tests. *Marinobacter alkaliphilus* strain (str)

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ODP1200D-1.5 was obtained from the Japan Collection of Microorganisms (JCM) for comparison. These organisms were studied more fully to ascertain their physiological limitations and preferences in terms of temperature, pressure, pH, sodium requirements for growth at high pH, and sodium chloride tolerance. Additionally, three of the isolates that showed exceptional characteristics were submitted to RTL genomics for library sequencing and subsequent comparative genomics.

MATERIALS AND METHODS

Media

Two main types of media were used in this research. Difco Marine Broth 2216 and

Alkaline MJYTGL medium pH 10.5 were used during isolation and high-pressure experiments

(Takei 2004). MJYTGL medium (MJ medium modified with the addition of yeast extract,

tryptone, glucose, and lactate) was used for the remaining characterization tests (Sako 1999,

Takei 2004). Alkaline MJYTGL medium includes $Na_2SiO_3 \times 9H_2O$, Na_2CO_3, and KOH, while

Standard MJYTGL medium does not (Takei 2004). The media was adjusted to 0.5 pH units

higher than the desired pH before autoclaving, as autoclaving was found to reduce the pH of the

media in all cases. The pH was checked and adjusted sterilely after autoclaving to ensure that the

desired pH was achieved. All pH adjustments to the media were made with filter-sterilized

potassium-hydroxide (KOH). For a list of the buffers used at each pH see Supplementary Table

2. Buffers were filter sterilized and added after autoclaving; the final concentration of buffer was

10mM in all cases. Modifications specific to each test, if necessary, are described in the

corresponding section of materials and methods.

Obtaining Isolates

During IODP Expedition 366 in 2017, cores were brought on deck, sectioned, and

sections designated for microbial analyses were removed with sterile spatulas and capped with

ethanol-rinsed plastic caps. Samples were quickly transported to a laminar flow hood in a 4°C

cold-room, where they were subsampled using a cut syringe, taking only the inner portion of

core sections to minimize contamination. These subsamples were then placed in 50mL centrifuge

tubes and stored in anaerobic bags at 4°C (Fryer et al. 2018). In 2018, these sediment samples

were plated out and allowed to incubate under a variety of conditions. Each sample was plated

onto Difco Marine Broth 2216 and Alkaline MJYTGL medium (pH 10.5) 1.7% agar plates. A plate of each type for each sample was then incubated at 5°C, 23°C, and 37°C under both aerobic and anaerobic conditions. Anaerobic conditions were achieved using the BD GasPak™ EZ Pouch System. Samples of drill fluid used during the collection of the sediment samples were plated and incubated under the same combinations of conditions. Any colonies that were observed on the plates were streaked for isolation and incubated under the same conditions as the parent plate. Isolated colonies were then selected for DNA extraction. The 16S rRNA gene of each isolate was amplified and sequenced using the conserved primers 27F and 1492R. The results were submitted to BLAST to obtain the closest cultured relative. Any matches above 97% were accepted as the closest sequence identity. A frozen stock of each isolate was prepared with 20% (v/v) glycerol and stored at -80°C. Isolates that were obtained from the drill fluid plates and the sediment plates were compared and any overlap was identified as possible contamination.

High Hydrostatic Pressure Incubation

Each isolate was grown up in its respective isolation medium aerobically in liquid culture and then inoculated into fresh liquid media at a 1:100 dilution. This fresh culture was used to fill 14 5mL plastic pipette bulbs at 6 pressures: 0.1MPa, 10 MPa, 20 MPa, 30 MPa, 40 MPa, and 50 MPa. The bulbs were placed into stainless steel, pin-closed pressure vessels with one uninoculated control per pressure vessel and pressurized (Yayanos 2001). The atmospheric pressure (0.1 MPa) cultures were incubated in opaque brown bottles filled with water to mimic the conditions of the pressure vessels. All incubations were at room temperature (23°C). The growth of the cultures was monitored via optical density (A600 nm) using GENESYSTM 10S UV-Vis Spectrophotometer, with 2 bulbs from each pressure taken at each time point and transferred into cuvettes.

Two exceptions to this protocol are the incubations of *Marinobacter alkaliphilus* str ODP1200D-1.5 and *Marinobacter alkaliphilus* str KM021. These cultures were incubated in Alkaline MJYTGL medium at a pH of 9, their optimum pH as determined from the pH incubations in this experiment, rather than in their isolation medium (Alkaline MJYTGL pH 10.5).

Further Characterization

All further incubations, unless otherwise noted, were performed in 10mL of MJYTGL medium in 15mL Hungate tubes sealed with a rubber stopper and crimp seal. This was done to prevent the pH of the MJYTGL medium from dropping with exposure to the atmosphere as a significant decrease in pH was observed in both inoculated and uninoculated aerobically incubated media. This drop in pH is predicted to be due carbon dioxide exchange between the air and the media. Sealing the Hungate tubes prevented changes in pH in uninoculated media, but does make the cultures technically oxygen limited. Starting cultures were inoculated into the Hungate tubes at a 1:100 dilution at the beginning of each characterization test using a needle and syringe. For pH 7 - 8.5 Standard MJYTGL medium was used, for pH 9-12 Alkaline MJYTGL medium was used. An uninoculated 15mL Hungate tube with 10mL of Alkaline MJYTGL medium pH 9 was used as a blank for all optical density measurements to ensure a consistent baseline. There was no significant difference observed in the optical density measurements of the uninoculated MJYTGL media pH 7-12. All conditions were performed and measured in triplicate unless otherwise noted.

pH Incubation

The isolates were grown up aerobically in Alkaline MJYTGL medium pH 10.5 and then inoculated into the Hungate tubes without washing. Each isolate was incubated in MJYTGL

medium pH 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, and 12. The cultures were incubated at 30°C in a shaking incubator with an uninoculated control for each pH and monitored via optical density measurements (A600 nm) using a Spectronic 20 Spectrophotometer.

Temperature Incubation

Each isolate was incubated in MJYTGL at its respective optimum pH, as determined from the pH incubations, in shaking incubators at 5°C, 17°C, 27°C, 37°C, 42°C, and 47°C. Cultures were monitored via optical density measurements (A600 nm) using a Spectronic 20 Spectrophotometer. An uninoculated control for each pH was incubated at each temperature. The estimated temperature *in situ* for each of the samples was calculated based on the published temperatures for the pressure and temperature at the subducting slab and at the seafloor (Fisher et al. 2020). A linear increase in temperature was assumed with depth, and the increased pressure due to the sediment was accounted for. All of the calculated estimates can be found in Supplementary Table 1.

Sodium Requirement Test

All sodium requirement incubations were performed in 10mL of MJYTGL medium made with no sodium (0% Na). Each isolate was grown up aerobically in 10mL of MJYTGL medium at its optimum pH, 1mL of each culture was then washed once with, and resuspended in, MJYTGL medium pH 7 0% Na. Each isolate was incubated at both pH 7 and pH 10 in a shaking incubator at 37°C with an uninoculated control for each pH. Cultures were monitored via optical density measurements (A600 nm) using a Spectronic 20 Spectrophotometer for 67 hours.

Two isolates were not included in this incubation because they did not grow at a pH of 10 in the pH incubations.

Sodium Chloride Tolerance Incubation

One representative isolate from each genus was tested for Sodium Chloride (NaCl) tolerance. The MJYTGL media for this test were made based on the NaCl tolerance experiment in Takei et al. 2004. The NaCl concentration of the MJ artificial seawater was used to determine the NaCl concentration of the medium, without accounting for Na^+ in other ingredients in the MJYTGL medium (Takei 2004). Using this method, MJYTGL medium was made with the following NaCl concentrations: 0%, 5%, 15%, and 25%. Each isolate was grown up aerobically in MJYTGL medium at its optimum pH and then washed once with, and resuspended in, MJYTGL pH 7 made with 0% Na^+ MJ artificial seawater. Each isolate was incubated in MJYTGL medium at its respective optimum pH, as determined from the pH incubations, in a shaking incubator at 37°C. Cultures were monitored via optical density measurements (A600 nm) using a Spectronic 20 Spectrophotometer.

Phylogenetic Trees

Phylogenetic trees were constructed for the selected isolates, separated by γ-Proteobacteria and Actinobacteria. Sequence alignment, model determination, and initial phylogenetic tree construction were performed in MEGA (Molecular Evolutionary Genetics Analysis) X (Kumar 2018). Trees were assembled using the Maximum Likelihood method. The Kimura 2-parameter model and Tamura-Nei model were used to construct the γ-Proteobacteria and Actinobacteria phylogenetic trees, respectively (Kimura 1980, Tamura and Nei 1993). The topology of the phylogenetic tree was determined using the Bootstrap method with 100 Bootstrap replications. Interactive Tree of Life was used for further processing of the phylogenetic trees (Letunic 2019).

Genomic Analyses

Three isolates were selected and sent to RTL Genomics for genome sequencing. Two of

the isolates, *Halomonas stevensii* str KM051 and *Halomonas johnsoniae* str KM073 were chosen

because they showed interesting results in physiological characterization tests. The third,

Marinobacter alkaliphilus str KM021, was included for comparative genomic analysis with the

Marinobacter alkaliphilus str ODP1200D-1.5 which was also sent for sequencing. Cultures were

grown up from frozen stock in liquid MJYTGL at the respective optimum pH of each isolate.

The cultures were then used to prepare a 10^5 dilution and plated onto MJYTGL 1.7% agar plates.

A single colony was selected for each isolate and DNA was extracted using the DNeasy

UltraClean Microbial Kit. Genomes were sequenced using the Illumina HiSeq sequencing

platform. The genomes were assembled using Velvet Assembler (v1.2.10) with a kmer length of

111 for *Halomonas johnsoniae* str KM073, and 99 for *Halomonas stevensii* str KM051,

Marinobacter alkaliphilus strain KM021, and *Marinobacter alkaliphilus* strain ODP1200D-1.5

(Zerbino and Birney 2008). The genomic analyses were performed using PATRIC and RASTtk

(v2.0) (Brettin et al. 2015, Wattam et al. 2017).

RESULTS

Plating

Plating under various conditions resulted in 159 isolates, 59 of which were isolated from plating samples of the drill fluid used during coring on the expedition. Sequencing of the 16S rRNA genes of these 59 isolates revealed 11 genera and 27 species. Two of these species, *Marinobacter hydrocarbonoclasticus* and *Halomonas shengliensis*, were also isolated from the sediment plates and therefore identified as possible contaminants. The conditions under which each of the ten isolates chosen for further characterization were isolated can be found in Supplementary Table 3.

Phylogenetic Analyses of Isolates

Sequencing of the16S rRNA genes of the isolates revealed that all of the organisms that were obtained in the plating were closely related to previously identified species and represented three genera: *Demequina, Halomonas,* and *Marinobacter*. Maximum likelihood trees based on the 16S rRNA genes were constructed for the ten isolates selected for further characterization; *Demequina salsinemoris* was compared with other species from the phylum Actinobacteria (Fig. 2) and the remaining isolates were compared with other species from the class γ-Proteobacteria (Fig. 3). These phylogenetic trees confirmed that all ten of the isolates were not novel species and that they grouped closely with their closest cultured relatives. Interestingly, some of the isolates grouped more closely with each other than with their closest cultured relatives. *Halomonas ventosae* str KM024 and *Halomonas shengliensis* str KM026 grouped more closely with each other than with their closest cultured relatives. *Halomonas hamiltonii* str KM072, *Halomonas johnsoniae* str KM073, and *Halomonas stevensii* str KM051 grouped more closely with each other than with their closest cultured relatives.
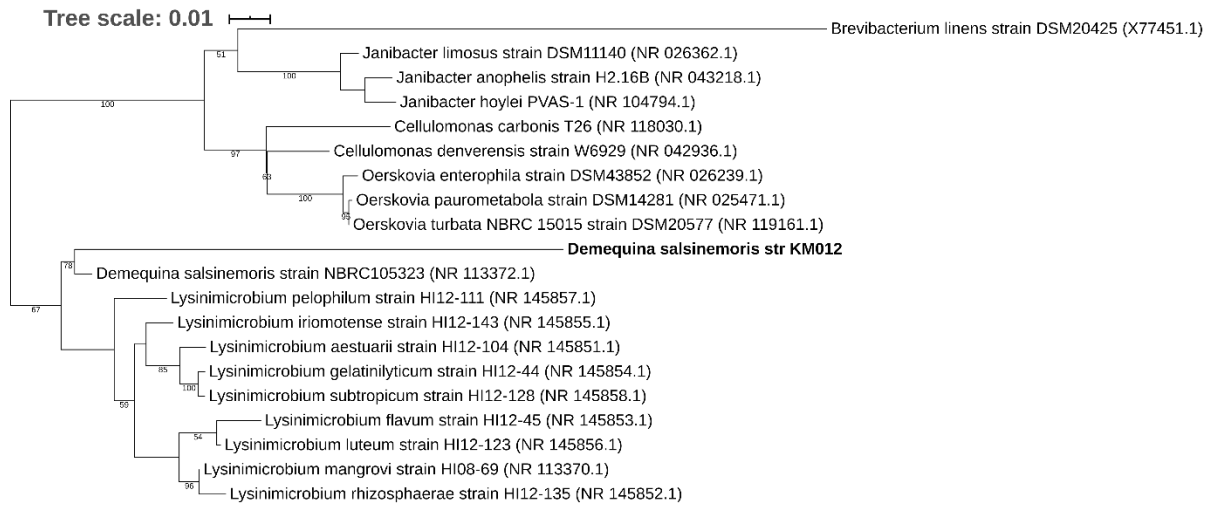
Figure 2. Actinobacteria Maximum Likelihood Phylogenetic Tree constructed using the Tamura-Nei Method. The tree is drawn to scale and branch lengths are measured in the number of substitutions per site. Bootstrap values (expressed as percentages of 100 replications) of greater than 50% are shown at branch points. GenBank accession numbers are shown in parathenses. Bar, 0.01 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA X and phylogenetic tree was edited using Interactive Tree of Life.

Figure 3. γ-Proteobacteria Maximum Likelihood Phylogenetic Tree constructed using the Kimura 2-parameter model. The tree is drawn to scale and branch lengths are measured in the number of substitutions per site. Bootstrap values (expressed as percentages of 100 replications) of greater than 50% are shown at branch points. GenBank accession numbers are shown in parathenses. Bar, 0.01 substitutions per nucleotide position. Evolutionary analyses were conducted in MEGA X and phylogenetic tree was edited using Interactive Tree of Life.

Pressure

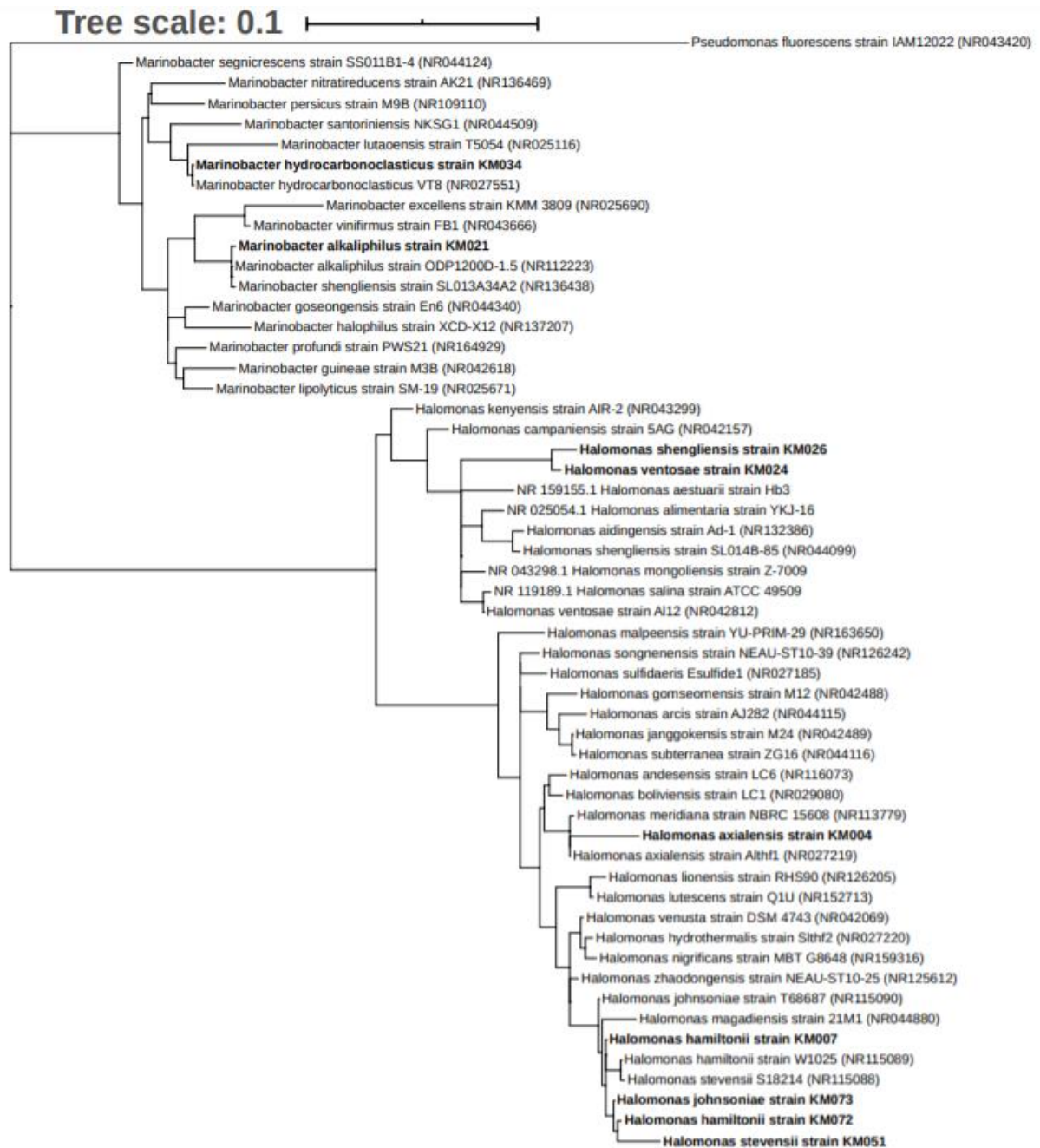The growth of each of the strains as a function of pressure was assessed in order to determine their adaptation to in situ pressure. Up to seven time points were taken for each isolate at each pressure, with fewer taken only if the cultures had reached stationary phase prior to the seventh time point. Reduced growth at atmospheric pressure (0.1MPa) compared to culture tube-based characterization tests can be attributed to the anoxic conditions that form in cultures grown in bulbs during incubation. All of the isolates and *Marinobacter alkaliphilus* str ODP1200D-1.5 exhibited optimal growth at atmospheric pressure. *Halomonas axialensis* str KM004 was able to grow at up to 40MPa, with a steady decline in growth rate with increasing pressure (Fig. 4A). *Halomonas ventosae* str KM024 and *Halomonas shengliensis* str KM026 exhibited very little growth even at atmospheric pressure and none at any of the increased pressures (Fig. 4B and G, Fig. 12B and G). *Halomonas hamiltonii* strains KM007 and str KM072, *Halomonas stevensii* str KM051, and *Halomonas johnsoniae* str KM073were able to grow at up to 40MPa with a steady decline in growth rate with increasing pressure (Fig. 4C, D, E, and F). *Demequina salsinemoris* str KM012 was able to grow at up to 40MPa, but with a reduced growth rate at 30MPa compared to 40MPa (Fig. 4H). The reason that this isolate did not grow well at 30MPa is unknown, but it is possible that the culture at 40MPa acquired an adaptive mutation to the high pressure conditions or that, although unlikely, an unobserved leak in the 40MPa pressure vessel decreased the actual pressure. *Marinobacter alkaliphilus* strain KM021 grew at up to 40MPa and *Marinobacter alkaliphilus* str ODP1200D-1.5 grew at up to 30MPa, with strain KM021 exhibiting a higher growth rate than strain ODP1200D-1.5 in all conditions (Fig. 5A-B). *Marinobacter hydrocarbonoclasticus* str KM034 was able to grow at up to 30MPa with a steady decline in

growth rate with increasing pressure (Fig. 5C). Growth curves for all isolates at all pressures can

be found in Supplementary Figures 1 and 2.
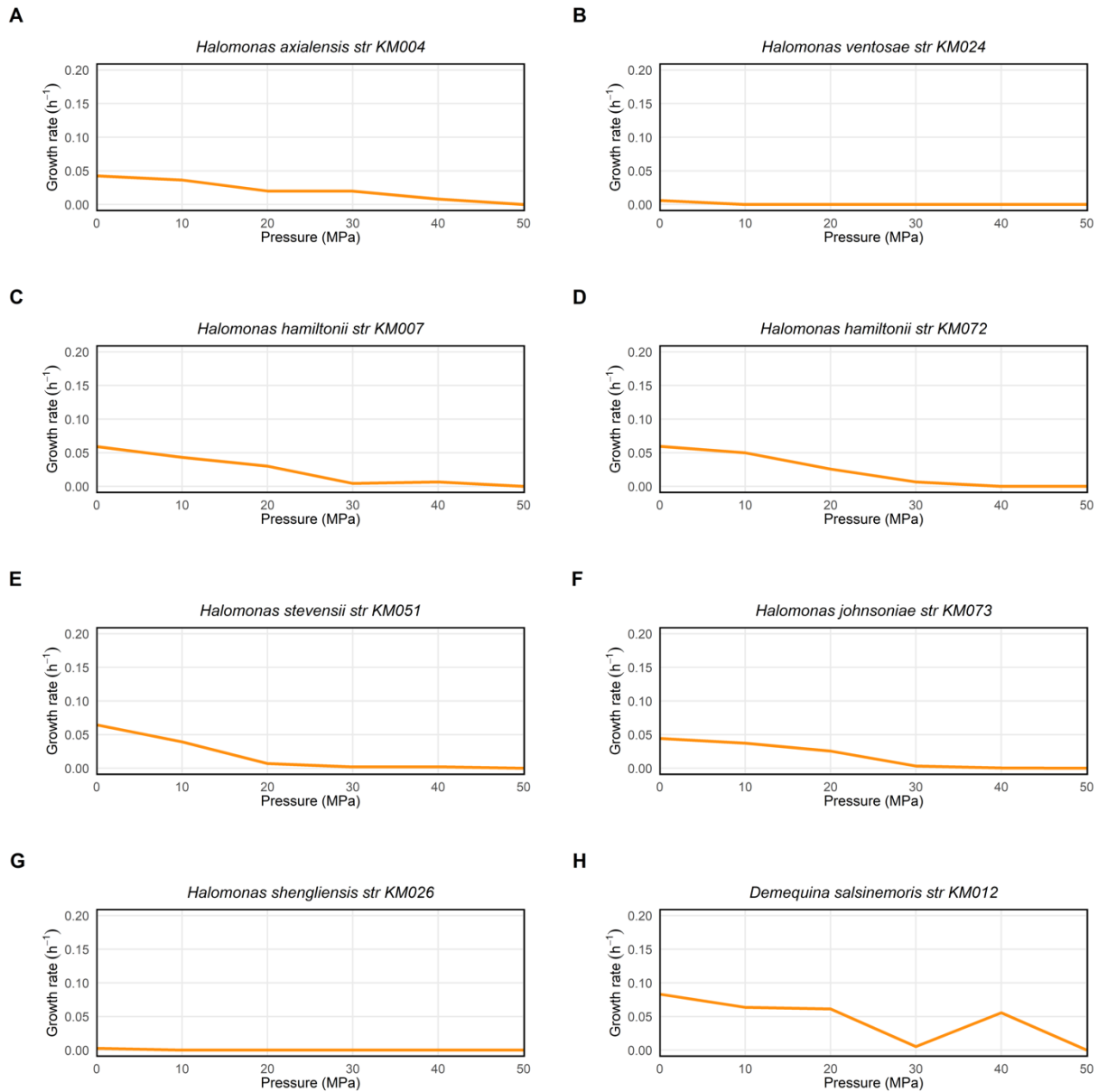
Figure 4. Effect of Pressure on the Specific Growth Rate of *Halomonas* and *Demequina* isolates. (A) *Halomonas axialensis* str KM004, incubated in MB2216, (B) *Halomonas ventosae* str KM024, incubated in MJYTGL medium pH 10.5, (C) *Halomonas hamiltonii* str KM007, incubated in MB2216, (D) *Halomonas hamiltonii* str KM072, incubated in MB2216, (E) *Halomonas stevensii* str KM051, incubated in MB2216, (F) *Halomonas johnsoniae* str KM073, incubated in MB2216, (G) *Halomonas shengliensis* str KM026, incubated in MJYTGL medium pH 10.5, (H) *Demequina salsinemoris* str KM012, incubated in MB2216. All incubations were performed at 23°C.

A

Marinobacter alkaliphilus str KM021

B

Marinobacter alkaliphilus str ODP1200D1.5

C

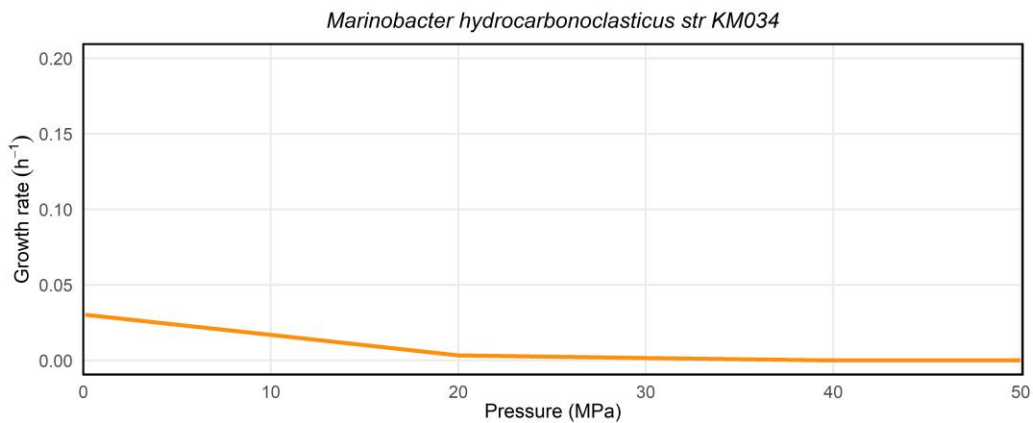*Marinobacter hydrocarbonoclasticus str KM034*
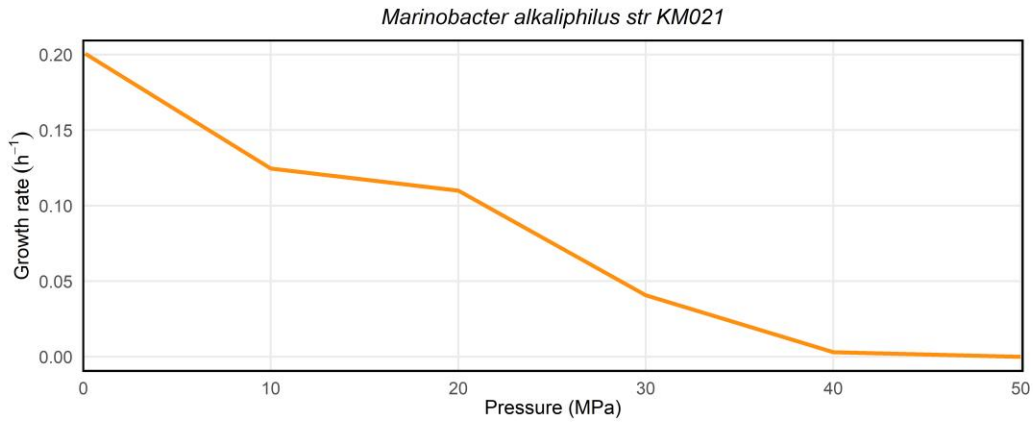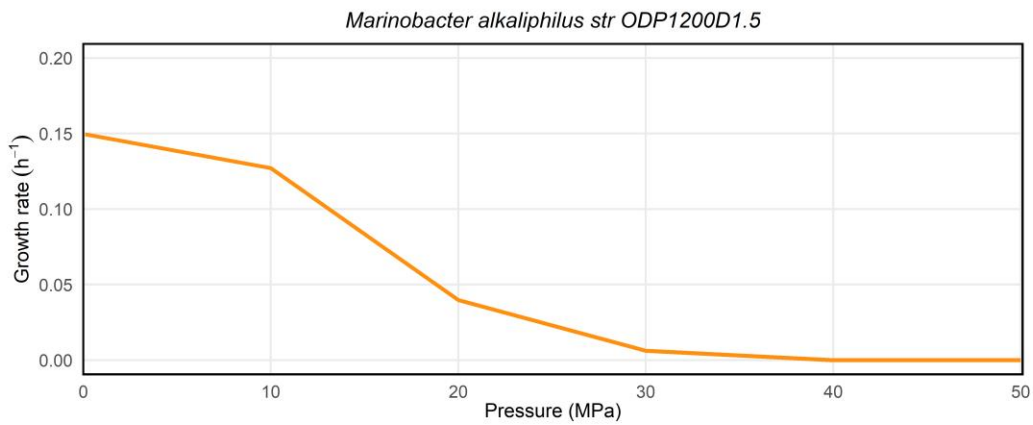
Figure 5. Effect of Pressure on the Specific Growth Rate of *Marinobacter* isolates. (A) *Marinobacter hydrocarbonoclasticus* str KM034, incubated in MB2216, (B) *Marinobacter alkaliphius* str KM021, incubated in MJYTGL medium pH 9, (C) *Marinobacter alkaliphilus* str ODP1200D-1.5, incubated in MJYTGL medium pH 9. All incubations were performed at 23°C.

pH

      In general, all of the isolates except for *Demequina salsinemoris* str KM012 exhibited at

least moderate alkalitolerance, growing up to a pH of at least 9.5. *Demequina salsinemoris* str

KM012 grew best at pH 7.5 and did not grow above pH 9, indicating that this strain is

mesophilic (Fig. 6H). *Halomonas axialensis* str KM004 is strongly alkalitolerant as it was able to

grow at a pH of up to pH 10.5 with optimal growth rate at pH 8.5 (Fig. 6H). *Halomonas ventosae*

str KM024 is alkalitolerant, as it was able to grow at a pH of up to 10 with optimal growth at pH

8.5 (Fig. 6B). *Halomonas hamiltonii* str KM007 is alkalitolerant as it was able to grow at a pH of

up to 10 with optimal growth at pH 7.5 (Fig. 6C). *Halomonas hamiltonii* str KM072 is

alkaliphilic, as it was able to grow at a pH of up to 11 with optimal growth at pH 9 (Fig. 6D).

The difference in the results between the two *Halomonas hamiltonii* strains is interesting and

could be due to the fact that they were isolated from different seamounts; strain KM007 was

isolated from sediment samples from Asùt Tesoru and strain KM072 was isolated from sediment

samples from Fantangisña. *Halomonas stevensii* strain KM051 is strongly alkalitolerant as it was

able to grow at a pH of up to 10.5 with optimal growth at pH 8.5 (Fig. 6E). Notably, the

differences in the specific growth rates between pH 8, 8.5, 9, and 10 for this strain are smaller

than 0.1 per hour (h^{-1}), with the growth at pH 9.5 slightly lower. *Halomonas johnsoniae* str

KM073 is alkaliphilic, as it was able to grow at a pH of up to 11 with optimal growth at pH 9

(Fig. 6F). *Halomonas shengliensis* str KM026 was alkalitolerant, as it was able to grow at a pH

of up to 10.5 with optimal growth at pH 8 (Fig. 6G). *Marinobacter alkaliphilus* str KM021 is

alkaliphilic as it was able to grow at a pH of up to 10 with optimal growth at both pH 9 and 10

(Fig. 7A). *Marinobacter alkaliphilus* strain ODP1200D-1.5, previously identified as alkaliphilic

by Takei et al., was able to grow at a pH of up to 10 with optimal growth at pH 8.5 (Fig. 7B).

This strain was previously described as being able to grow up to a pH of approximately 11.5 with

optimal growth at approximately pH 9, however, those experiments were conducted differently than in this research which could account for the discrepancy (Takei et al. 2005). The differences in the results between the two strains are not large, but *Marinobacter alkaliphilus* str KM021 did generally grow more slowly than strain ODP1200D-1.5. It is possible that the differences are due to the fact that they were isolated from different seamounts and are therefore differently adapted. *Marinobacter hydrocarbonoclasticus* strain KM034 was moderately alkalitolerant, as it was able to grow at a pH of up to 9.5 with optimal growth at pH 7(Fig. 7C). Growth Curves for all isolates at all pHs can be found in Supplementary Figures 3 and 4.

Figure 6. Effect of pH on the Specific Growth Rate of *Halomonas* and *Demequina* isolates. (A) *Halomonas axialensis* str KM004, (B) *Halomonas ventosae* str KM024, (C) *Halomonas hamiltonii* str KM007, (D) *Halomonas hamiltonii* str KM072, (E) *Halomonas stevensii* str KM051, (F) *Halomonas johnsoniae* str KM073, (G) *Halomonas shengliensis* str KM026, (H) *Demequina salsinemoris* str KM012. All incubations were performed in MJYTGL medium at 30°C in a shaking incubator

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Figure 7. Effect of pH on the Specific Growth Rate of *Marinobacter* isolates. (A) *Marinobacter hydrocarbonoclasticus* str KM034, (B) *Marinobacter alkaliphilus* str KM021, (C) *Marinobacter alkaliphilus* str ODP1200D-1.5. All incubations were perfomed in MJYTGL medium at 30°C in a shaking incubator.

Temperature

The majority of the isolates grew over a broad temperature range, from 5°C to 47°C, and exhibited a decreased growth rate at 47°C. *Demequina salsinemoris* str KM012 and *Marinobacter alkaliphilus* str ODP1200D-1.5 were not able to grow at 5°C. While eight of the eleven strains experienced optimum growth rates at 42°C, in all but one case they did not reach as high of an optical density at this temperature as they did at lower temperatures (Fig. 8A-G, Fig. 16A-G). Interestingly, all of these strains achieved their highest optical densities at either 5°C or 17°C, as did *Marinobacter alkaliphilus* str ODP1200D-1.5 and *Demequina salsinemoris* str KM012 despite the fact their optimum growth rates were at much higher temperatures (Fig. 16 and 17, blue lines). The exception to this result is *Marinobacter alkaliphilus* str KM021, which achieved its highest optical densities at 42°C and 47°C, with optimal growth at 42°C (Fig. 9A). *Marinobacter alkaliphilus* str ODP1200D-1.5, previously identified as growing optimally at 30°C, had its highest growth rate in this study at 37°C (Fig. 9B) (Takei et al. 2005). *Marinobacter hydrocarbonoclasticus* strain KM034 and *Demequina salsinemoris* str KM012 also had optimal growth rates at 37°C (Fig. 9C and 8H). Growth curves for all isolates at all temperatures can be found in Supplementary Figures 5 and 6.
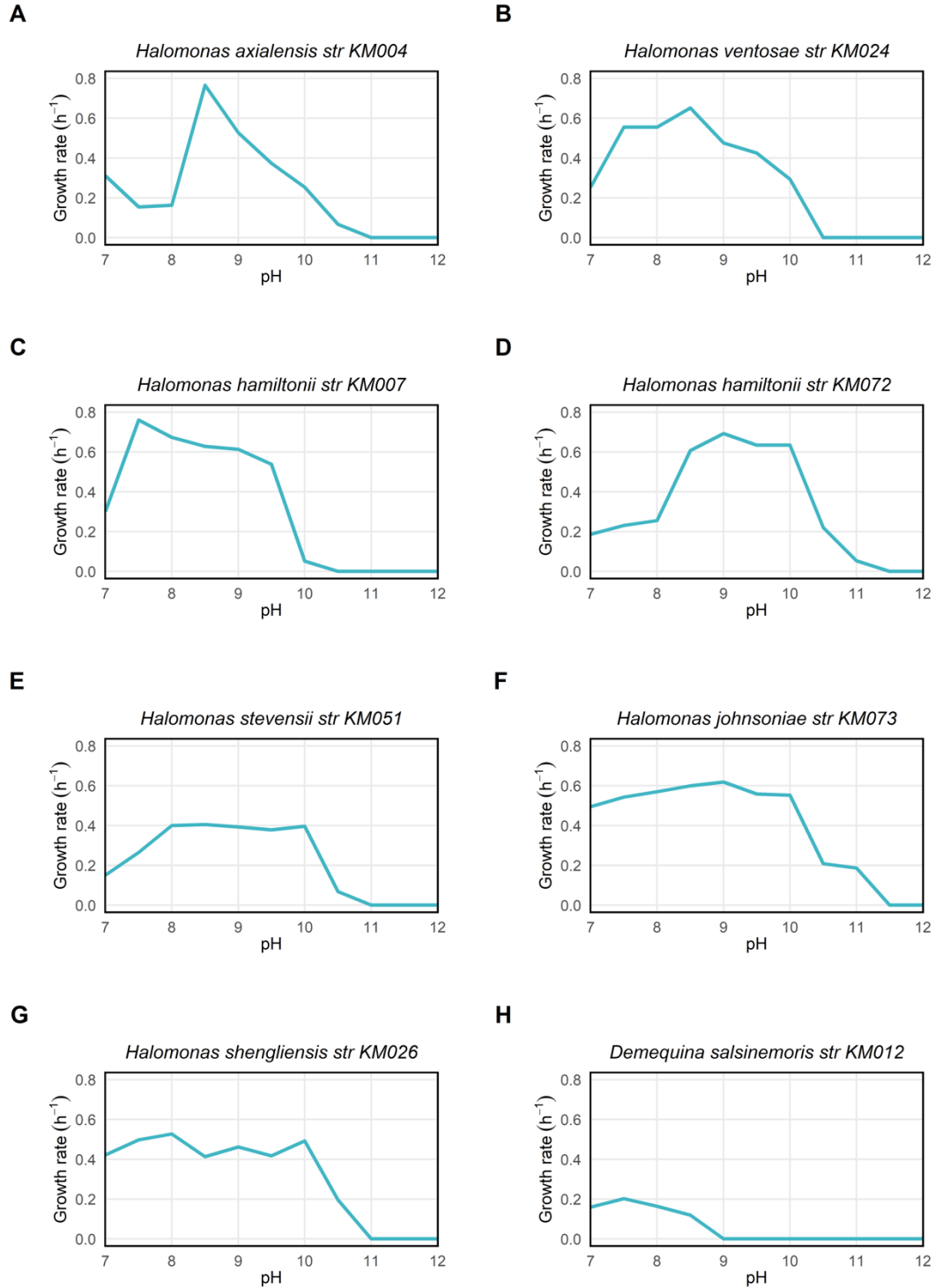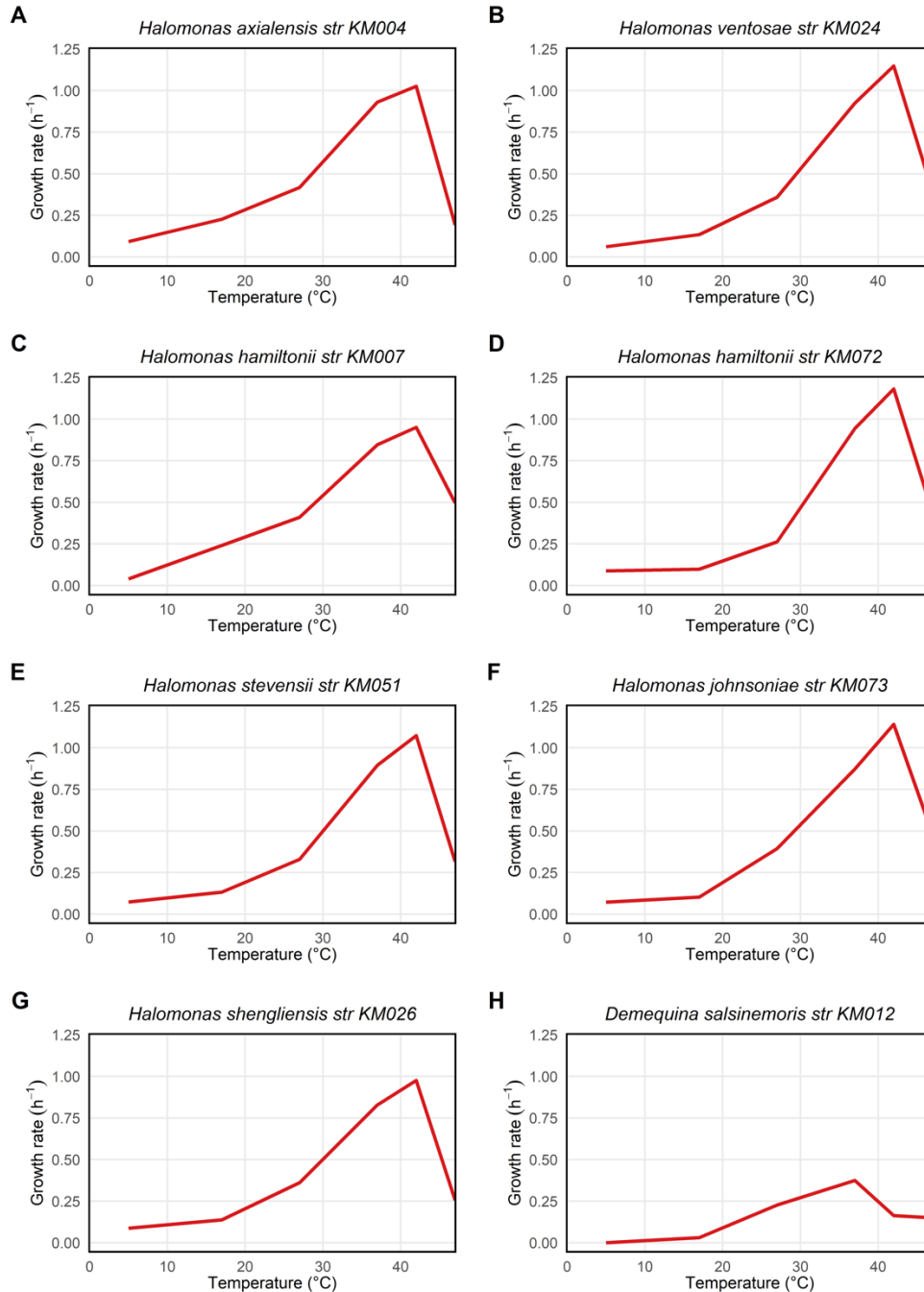
Figure 8. Effect of Temperature on the Specific Growth Rate of *Halomonas* and *Demequina* isolates. (A) *Halomonas axialensis* str KM004, incubated MJYTGL medium 8.5, (B) *Halomonas ventosae* str KM024, incubated in MJYTGL medium pH 8.5, (C) *Halomonas hamiltonii* str KM007 incubated in MJYTGL medium pH 7.5, (D) *Halomonas hamiltonii* str KM072, incubated in MJYTGL medium pH 9, (E) *Halomonas stevensii* str KM051, incubated in MJYTGL medium pH 8.5, (F) *Halomonas johnsoniae* str KM073, incubated in MJYTGL medium pH 9, (G) *Halomonas shengliensis* str KM026, incubated in MJYTGL medium pH 8, (H) *Demequina salsinemoris* str KM012, incubated in MJYTGL medium pH 7.5. All incubations were performed in shaking incubators.

A

Marinobacter alkaliphilus str KM021

B

Marinobacter alkaliphilus str ODP1200D1.5
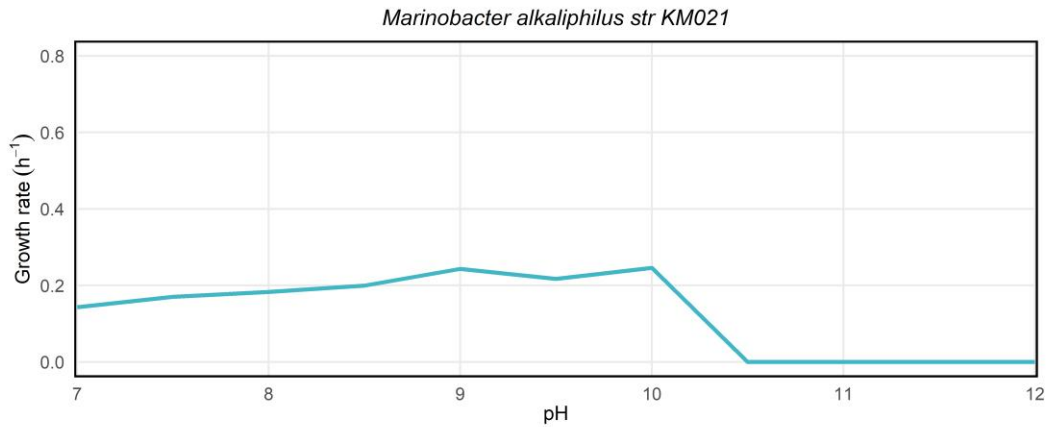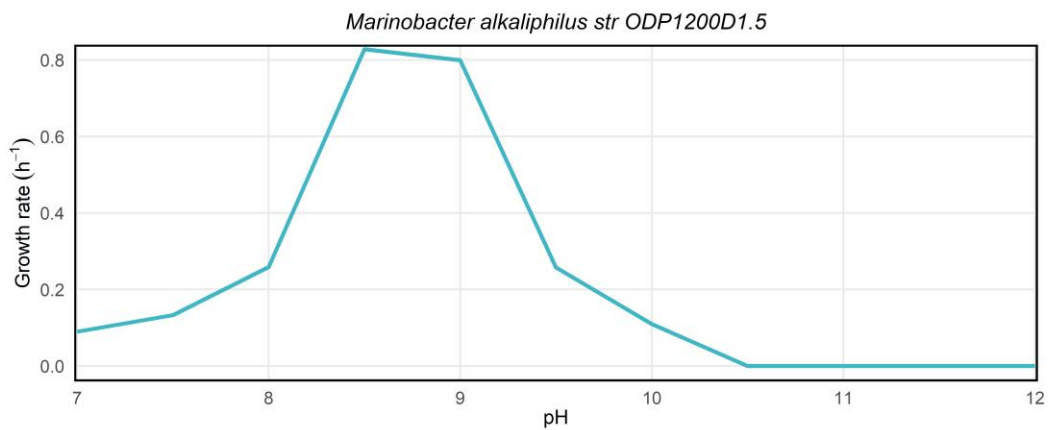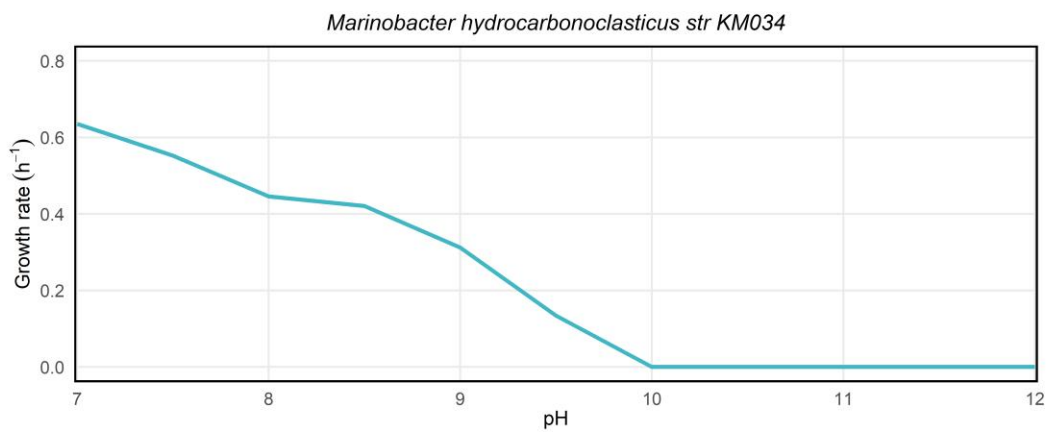
C

Marinobacter hydrocarbonoclasticus str KM034
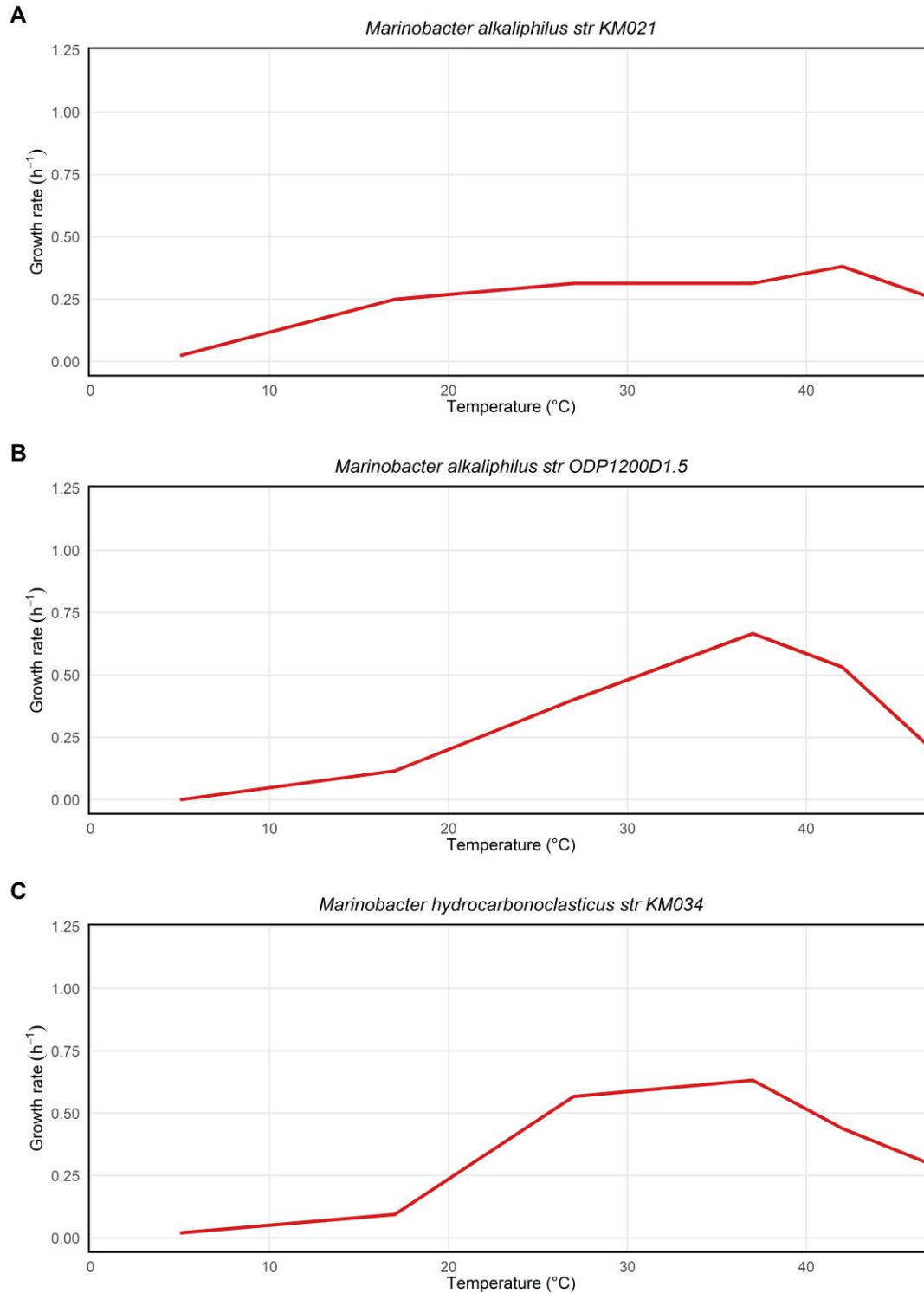
Figure 9. Effect of Temperature on the Specific Growth Rate of *Marinobacter* isolates. (A) *Marinobacter hydrocarbonoclasticus* str KM034, incubated in MJYTGL medium pH 7, (B) *Marinobacter alkaliphilus* str KM021, incubated in MJYTGL medium pH 9, (C) *Marinobacter alkaliphilus* str ODP1200D-1.5, incubated in MJYTGL medium pH 9. All incubations were performed in shaking incubators.

NaCl Tolerance

Only 4 strains were included in this analysis, one representative strain from each genus as well as *Marinobacter alkaliphilus* str ODP1200D-1.5. All of the strains experienced a reduction in growth rate with increased NaCl concentration, and *Demequina salsinemoris* str KM012 could not grow in the MJYTGL medium made with 15% NaCl MJ artificial seawater (Fig. 10B). The remaining three strains had optimal growth in the MJYTGL medium made with 0% NaCl artificial seawater, and could grow in media made with up to 20% NaCl (Fig. 10A, C, D). *Marinobacter alkaliphilus* str ODP1200D-1.5 was previously characterized via this test as having an NaCl optimum of 2.5-3.5% (Takei et al. 2005); these concentrations were not included in the experiments done as part of this research (Takei et al. 2005). Growth curves for all four strains at all concentrations can be found in Supplementary Figure 7.
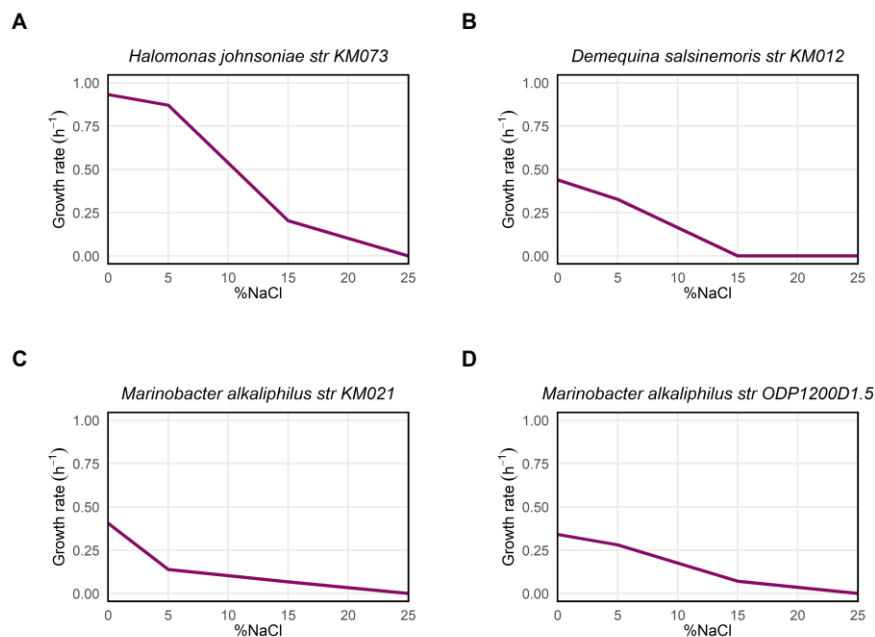
Figure 10. Effect of NaCl Concentration on Specific Growth Rate. (A) *Halomonas johnsoniae* str KM073, incubated in MJYTGL medium pH 9, (B) *Demequina salsinemoris* str KM012, incubated in MJYTGL medium pH 7.5, (C), *Marinobacter alkaliphilus* str KM021, incubated in MJYTGL medium pH 9, (D) *Marinobacter alkaliphilus* str ODP1200D-1.5, incubated in MJYTGL medium pH 9. The MJYTGL media contained a varying concentration of NaCl from 0% - 25% instead of 3% NaCl. All incubations were performed at 37°C in shaking incubators.

Sodium Requirement

Since many alkaliphiles require at least some sodium for growth at high pH, the need for sodium was also investigated. Two isolates, *Demequina salsinemoris* str KM012 and *Marinobacter hydrocarbonoclasticus* str KM034, were excluded from these experiments because they were found not to grow at high pH in the pH characterization tests. The results for the other strains are presented in Figure 11 and Table 1. In general, the isolates experienced reduced growth in the no sodium media, and under these conditions were not able to grow at pH 10. *Halomonas shengliensis* str KM026 experienced the smallest reduction in growth at pH 7, but was still severely stunted compared to growth in pH 7 in the standard medium employed (MJYTGL). The one exception to this generalized result is *Halomonas stevensii* strain KM051 which, interestingly, experienced increased growth at pH 10 compared to pH 7.
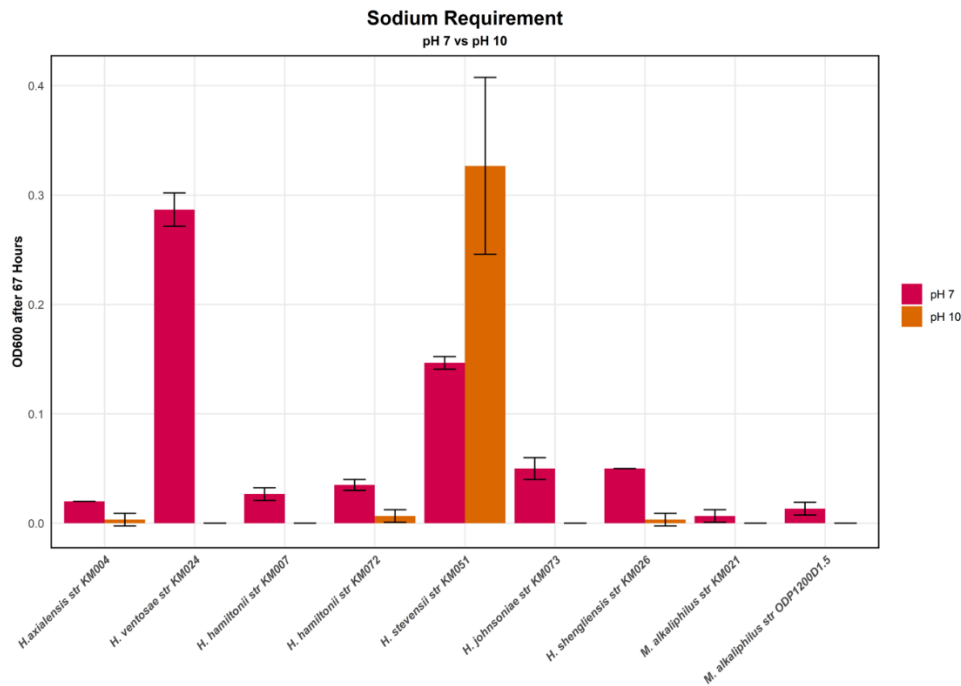
Figure 11. Determination of Sodium Requirement for Nine Isolates. All incubations were performed at 30°C in a shaking incubator for 67 hours. An OD was taken at time 0 and at 67 hours. Errors bars represent the standard deviation calculated from the results of 3 replicates. The MJYTGL medium pH 7 and pH 10 were made by omitting all ingredients that contained Na. A trace amount ($< 3\mu$M) of Na could not be eliminated from a pre-prepared trace element solution

Table 1. Growth in Zero Sodium MJYTGL Media

Isolate	pH 7 0% Na	pH 10 0% Na
Halomonas axialensis str KM004	✔ Yes	✘ No
Halomonas ventosae str KM024	✔ Yes	✘ No
Halomonas hamiltonii str KM007	✔ Yes	✘ No
Halomonas hamiltonii str KM072	✔ Yes	✘ No
Halomonas stevensii str KM051	✔ Yes	✔ Yes
Halomonas johnsoniae str KM073	✔ Yes	✘ No
Halomonas shengliensis str KM026	✔ Yes	✘ No
Marinobacter alkaliphilus str KM021	✔ Yes	✘ No
Marinobacter alkaliphilus str ODP1200D1.5	✔ Yes	✘ No

Genomic Analyses

The four strains analyzed - *Halomonas johnsoniae* str KM073, *Halomonas stevensii* strain KM051, *Marinobacter alkaliphilus* str KM012, and *Marinobacter alkaliphilus* str ODP1200D-1.5 – shared a number of metabolic pathways and genes that could contribute to survival in a serpentinizing system.

Table 2. Summary of Genome Features of *Halomonas stevensii* str KM051, *Halomonas johnsoniae* str KM073, *Marinobacter alkaliphilus* str KM021, and *Marinobacter alkaliphilus* str ODP1200D-1.5.

Characteristic	Halomonas stevensii str KM051	Halomonas johnsoniae str KM073	Marinobacter alkaliphilus str KM021	Marinobacter alkaliphilus str ODP1200D-1.5
Contigs	229	172	243	135
GC Content (%)	59.56	57.37	57.29	60.11
Contig L50	15	16	19	14
Contig N50	82,256	85,282	72,066	83,603
Genome Length (bp)	3,796,052	4,062,052	4,484,303	3,554,719
CDS	3,736	3,836	4,343	3,469
Repeat Regions	88	53	50	85
tRNA	58	51	18	58
rRNA	7	6	8	9

The draft genome of *Halomonas johnsoniae* str KM073 (Supplementary Fig. 8) is comprised of 172 contigs and is 4,062,052 base pairs (bp). It has an average G+C content of

57.37% (Table 2) and was shown to have 3,836 coding sequences. These coding sequences include 1,006 hypothetical proteins and 2,830 proteins with functional assignments. The genome encodes 51 tRNAs, 6 rRNAs, 9 transposon genes, 53 CRISPR repeats, 52 CRISPR spacers, and 1 CRISPR array. Genomic analysis indicates that this microbe is a heterotroph capable of using diverse forms of organic carbon including aromatic compounds, and respiring oxygen, nitrate, and nitrite. Several metabolic pathways that could allow this species to survive in the serpentinite seamounts of the forearc were identified. Six genes representing the three subunits of formate dehydrogenase are present, suggesting that formate could be utilized as a source of carbon and energy. Many of the genes in the propionate metabolism pathway were also identified. The presence of one acetyl-coA synthetase gene and the components of the glyoxylate bypass indicate that this species could be capable of metabolizing acetate (Cozzone 1998, De Mets et al. 2019). Genes that could contribute to the survival in alkaline environment of the seamounts include many sodium-dependent transporters and genes for compatible solutes such as ectoine (Czech et al. 2018, Williamson et al. 2016). Additionally, this species has at least two complete gene sets for a group 1 Mrp system and one complete gene set for a group 2 Mrp system. The Mrp system is believed to be the dominant Na^+/H^+ antiporter for pH homeostasis in alkaliphilic species and has a role in Na^+ resistance (Hiramatsu et al. 1998, Kitada et al. 2000, Krulwich et al. 2007, Swartz et al. 2005). A number of genes that have been associated with piezophily and piezotolerance were also identified, such as five heat and five cold shock proteins and the subunits of the cbb3 cytochrome c oxidase (Chikuma et al. 2007, Liu et al. 2020, Simonato et al. 2006). While these proteins are important in many processes, they have been found to be present in most piezophiles and have been implicated in their adaptative mechanisms.

The draft genome of *Halomonas stevensii* str KM051 (Supplementary Fig. 9) is comprised of 229 contigs and is 3,796,052bp. It has an average G+C content of 59.56% (Table 2) and was shown to have 3,742 coding sequences. These coding sequences include 937 hypothetical proteins and 2,799 proteins with functional assignments. The genome encodes 58 tRNAs, 7 rRNAs, 8 transposons, 88 CRISPR repeats, 87 CRISPR spacers, and 1 CRISPR array. Genomic analysis indicates that this microbe is a heterotroph capable of using diverse forms of organic carbon, including aromatic compounds, and respiring oxygen, nitrate, and nitrite. There is also evidence that it is capable of tetrathionate reduction, which is not present in the published genome of *Halomonas stevensii* str S18214 (Kim et al. 2012). This species shared the metabolic pathways described above for *Halomonas johnsoniae* str KM073 that could allow it to survive in a serpentinizing system, with some differences in the number of gene copies. Four genes representing the three subunits of formate dehydrogenase o, and two acetyl-coA synthetase genes are present. The genes required for the enzyme urease are also present in the genome, which has been identified in microbes isolated from other serpentinizing environments (Suzuki et al. 2014). Many genes that could contribute to survival in the alkaline environment of the seamounts are shared with *Halomonas johnsoniae* str KM073 and are described above. Additionally, two NhaD-type antiporters, which have been previously linked to alkaliphiles, are present in the genome (Kurz et al. 2006, Liu et al. 2005, Nozaki et al. 1998). One complete set of genes for a group 1 Mrp system are also present (Hiramatsu et al. 1998, Kitada et al. 2000, Krulwich et al. 2007, Swartz et al. 2005). A number of genes that have been associated with piezophily and piezotolerance were also identified, such as six heat and four cold shock proteins and the subunits of the cbb3 cytochrome c oxidase (Chikuma et al. 2007, Liu et al. 2020, Simonato et al. 2006)

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The draft genome of *Marinobacter alkaliphilus* str KM021 (Supplementary Fig. 10) is comprised of 243 contigs and is 4,484,303bp. It has an average G+C content of 57.28% (Table 2) and was shown to have 4,346 coding sequences. These coding sequences include 1,118 hypothetical proteins and 3,225 proteins with functional assignments. The genome encodes 50 tRNAs, 8 rRNAs, 1 transposon gene, 18 CRISPR repeats, 17 CRISPR spacers, and 1 CRISPR array. Genomic analysis indicates that this microbe is a heterotroph capable of using diverse forms of organic carbon, including aromatic compounds, respiring oxygen and nitrate, and denitrification. This species shared the metabolic pathways described above for *Halomonas johnsoniae* str KM073 that could allow it to survive in a serpentinizing system. Many genes that could contribute to survival in the alkaline environment of the seamounts are shared with *Halomonas johnsoniae* str KM073 and are described above. Additionally, this species has at least two complete gene sets for a group 1 Mrp system and one complete gene set for a group 2 Mrp system. A number of genes that have been associated with piezophily and piezotolerance were also identified, such as six heat and five cold shock proteins and the subunits of the cbb3 cytochrome c oxidase (Chikuma et al. 2007, Liu et al. 2020, Simonato et al. 2006)

The draft genome of *Marinobacter alkaliphilus* str ODP1200D-1.5 (Supplementary Fig. 11) is comprised of 135 contigs and is 3,554,719bp. It has an average G+C content of 60.11% (Table 2) and was shown to have 3,471 coding sequences. These coding sequences include 934 hypothetical proteins and 2,535 proteins with functional assignments. The genome encodes 58 tRNAs, 9 rRNAs, 81 transposase genes, 85 CRISPR repeats, 83 CRISPR spacers, and 2 CRISPR arrays. Genetic analysis indicates that this microbe is capable of using diverse forms of organic carbon, respiring oxygen, utilizing nitrogen, catabolizing aromatic compounds, and metabolizing central aromatic intermediates. This species shared the metabolic pathways described above for

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Halomonas johnsoniae str KM073 that could allow it to survive in a serpentinizing system, with some differences in the number of gene copies. Four genes representing the three subunits of formate dehydrogenase o and two acetyl-coA synthetase genes are present. The genes required for the enzyme urease are also present in the genome (Suzuki et al. 2014). Many genes that could contribute to survival in the alkaline environment of the seamounts are shared with *Halomonas johnsoniae* str KM073 and are described above. Additionally, two NhaD-type antiporters are present (Kurz et al. 2006, Liu et al. 2005, Nozaki et al. 1998, Williamson et al. 2016). Interestingly, the NhaD-type antiporters are not present in the draft genome of *Marinobacter alkaliphilus* str KM021. One complete set of genes for a group 1 Mrp system are also present (Hiramatsu et al. 1998, Kitada et al. 2000, Krulwich et al. 2007, Swartz et al. 2005). A number of genes that have been associated with piezophily and piezotolerance were also identified, such as eight heat and four cold shock proteins and the subunits of the cbb3 cytochrome c oxidase (Chikuma et al. 2007, Liu et al. 2020, Simonato et al. 2006)

DISCUSSION

The microbial communities at serpentinite seamounts are poorly understood and only one species isolated from these environments, *Marinobacter alkaliphilus*, had been characterized prior to this study (Takei et al. 2005). Following the plating of unamended sediment samples from three seamounts, 100 isolates were obtained. These isolates represented four sediment samples from two seamounts, Asùt Tesoru and Fantangisña. A subset of ten isolates was chosen for further characterization and analysis. The identification and characterization of these ten isolates contributes to the knowledge of the microbial communities, and their physio-chemical adaptations, at serpentinite seamounts. All of the isolates obtained in this study were closely related to previously identified species, but many were able to grow at the elevated pH and reduced temperatures that microbes would experience *in situ*. A summary of the results for each isolate can be found in Table 3.

Isolate Descriptions

Sequencing of the 16S rRNA gene and construction of phylogenetic trees for the ten isolates show that they do not represent novel species. All of the isolate's 16S rRNA genes were at least 98% identical to previously identified species, and no monophyletic branches were formed in the phylogenetic trees. This confers a high degree of confidence that the species designations are correct. The 16S rRNA gene sequencing results are as follows. *Halomonas axialensis* str KM004 shares 99.48% identity with *Halomonas axialensis* str Althf1 (Kaye et al. 2004). *Halomonas ventosae* str KM024 shares 98.27% identity with *Halomonas ventosae* str Al12 (Martinez-Canovas et al. 2004). *Halomonas hamiltonii* strains KM007 and KM072 share 99.34% and 99.19% identity, respectively, with *Halomonas hamiltonii* str W1025 (Kim et al. 2010). *Halomonas stevensii* str KM051 shares 98.89% identity with *Halomonas stevensii* str

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S18214 (Kim et al. 2010). *Halomonas johnsoniae* str KM073 shares 99.57% identity with *Halomonas johnsoniae* str T68687 (Kim et al. 2010). *Halomonas shengliensis* str KM026 shares 98.68% identity with *Halomonas shengliensis* str SL014B-85 (Wang et al. 2007). *Demequina salsinemoris* str KM012 shares 98.56% identity with *Demequina salsinemoris* str NBRC 105323 (Matsumoto et al. 2010). *Marinobacter alkaliphilus* str KM021 shares 99.56% identity with *Marinobacter alkaliphilus* str ODP1200D-1.5 (Takei et al. 2005). *Marinobacter hydrocarbonoclasticus* str KM034 shares 99.79% identity with *Marinobacter hydrocarbonoclasticus* str VT8 (Huu et al. 1999).

Three genera were represented in the isolates obtained from plating out the sediment samples at atmospheric pressure: *Halomonas, Demequina, and Marinobacter.* The genus *Halomonas*, a member of the family *Halomonadaceae* and the class γ-Proteobacteria, is virtually omnipresent and has been found in a wide variety of saline environments from hydrothermal vents to the interior of dialysis machines (Kim et al. 2010, Vreeland 2015). Members of the gram-negative genus are often described as halotolerant, and some species are alkaliphilic (Kaye et al. 2004, Kim et al. 2010, Vreeland 2015). The genus *Marinobacter*, a member of the family *Alteromonadaceae* and the class γ-Proteobacteria, was established in 1992 (Gauthier et al. 1992). This gram-negative genus is found throughout the marine environment and in many other saline environments (Kim et al. 2017). Members of this genus are described as halophilic and many have been shown to be capable of degrading hydrocarbons (Gauthier et al. 1992, Brito et al. 2006, Kim et al. 2017). The genus *Demequina*, a taxonomic synonym to *Lysinimicrobium,* was established in 2007 and is a member of the family *Demequinaceae* and the phylum Actinobacteria (Yi et al. 2007, Ue et al 2011). This gram-positive genus includes many species that synthesize unique molecules (Servin et al., 2007, Park et al. 2015). The *Demequina* are

named for one such unique molecule, demethylmenaquinone (Hamada et al. 2013, Ue et al. 2011, Yi et al. 2007). This genus is found in both aquatic and terrestrial environments from marine sediments to mangrove soils, is not known to be halotolerant, and is generally neutralophilic (Hamada et al. 2013, Matsumoto et al. 2010, Park et al. 2015, Yi et al. 2007).

Of the ten isolates examined in this research only two species, *Halomonas axialensis* str KM004 and *Marinobacter alkaliphilus* str KM021, corresponded to species previously isolated from marine environments. *Halomonas axialensis* was first isolated in 2004 from a low-temperature hydrothermal vent in the Juan de Fuca ridge (Kaye et al. 2004). This environment is quite similar to the Mariana Forearc seamounts in that the underlying mantle is highly serpentinized and thus the resulting chemical environments are alike (Schiffman et al. 2010). Prior to this research, *Marinobacter alkaliphilus* had only been isolated once, from sediment cores taken during ODP Leg 195 at South Chamorro Seamount in the Mariana Forearc (Takai et al. 2005). Of the seamounts sampled in IODP expedition 366, South Chamorro seamount is most similar to Asùt Tesoru, from the sediment of which *Marinobacter alkaliphilus* str KM021 was isolated (Salisbury et al. 2002, Fryer et al. 2020). The seamounts share distance to trench, depth to slab, pore water pH, and have similar slab temperatures and chemical compositions (Fryer et al. 2020). The presence of *Marinobacter hydrocarbonoclasticus* str KM034, a species originally isolated from an oil wellhead, in the sediment samples may be a result of drill fluid contamination, as it was also isolated from plating samples of the drill fluid used during IODP Expedition 366 (Fryer et. al 2020a, Huu et al. 1999). It is useful, however, as a comparison to *Marinobacter alkaliphilus* strains KM021 and ODP1200D-1.5. Four of the isolates, *Halomonas johnsoniae* str KM073, *Halomonas stevensii* str KM051, and *Halomonas hamiltonii* strains KM004 and KM072, comprise three species that were first discovered together in 2010 in a

dialysis unit at a renal care center (Kim et al. 2010). While at first glance this seems like an indication that these strains do not belong in a marine sediment sample, all three strains share between 98 and 98.5% 16S rRNA gene sequence similarity with marine strains such *Halomonas axialensis* and *Halomonas aquamarina* (Kim et al. 2010) and were incubated in media containing sea salts throughout their original characterization. The two remaining *Halomonas* species, *Halomonas ventosae* str KM024 and *Halomonas shengliensis* str KM026, both represent species first isolated in saline soils, from Spain and China, respectively (Martínez-Cánovas et al. 2004, Wang et al. 2007). The final isolate, *Demequina salsinemoris* str KM012, was the only *Demequina* species identified in this research; this species was isolated from mangrove soil on an island off the coast of Japan (Matsumoto et al. 2010).

Physical-chemical Characterizations

All ten of the isolates as well as *Marinobacter alkaliphilus* str ODP1200D-1.5 are characterized as being pressure sensitive; none of the species that were examined in this research are piezophilic and none have more than a moderate piezotolerance. The three seamounts in this study, Asùt Tesoru, Fantangisña, and Yinazao, are not located at extreme depths, with a maximum sample depth of approximately 3.4km corresponding to about 34MPa. The majority of the isolates stopped showing signs of growth at 30MPa, but had measurable growth rates at 10MPa and 20MPa. It is therefore feasible that there could be active communities shallower in the sediment on the summits and flanks of the seamounts, which can be located less than 1,250mbsl (Fryer et al. 2018b).

The majority of the isolates are alkalitolerant and some are alkaliphilic. *Halomonas johnsoniae* str KM073 was able to grow over the largest range of pH, up to 11.5, with optimal growth at pH 9. *Marinobacter alkaliphilus* str KM021 had the highest optimum growth rate, pH

10, and two other isolates exhibited relatively steady growth rates between pH 8 and pH 10. The growth ranges and optima of the isolates generally corresponded to the published values for the most closely related species, with a few notable exceptions. *Halomonas axialensis* str KM004, *Halomonas hamiltonii* str KM072, and *Halomonas johnsoniae* str KM073 exhibited pH optima at least 0.5 pH units higher than published for another member of their respective species (Kaye et al. 2004, Kim et al. 2010). *Marinobacter alkaliphilus* str ODP1200D-1.5 exhibited markedly more robust growth than *Marinobacter alkaliphilus* str KM021. This is an interesting result and may be elucidated by genomic comparisons. The serpentinization reaction creates an alkaline environment, and some of the seamounts have pH values above 12.5, the current known limit of life (Takai et al. 2001). It has been hypothesized that microbial communities at serpentinite mud volcanoes should be dominated by alkaliphiles because of the high pHs created during serpentinization that occur throughout subduction and re-emergence (Fryer et al. 2020).

All of the isolates were able to grow at a broad temperature range, but showed an optimum at either 37°C or 42°C. This corresponds with the existing literature, as the closest cultured relatives of all of the isolates were characterized as mesophilic (Huu et al. 1999, Kaye et al. 2004, Kim et al. 2010, Martínez-Cánovas et al. 2004, Matsumoto et al. 2010, Schiffman et al. 2010, Takai et al. 2005, Wang et al. 2007,). While any microbes that experience subduction or live deep in the sediment would be subject to temperatures up to 350°C, the summits of the seamounts are generally the same temperature as the seafloor. The estimated *in-situ* temperatures for the microbes analyzed in this study are between 1.7°C and 3.7°C. While none of the isolates had growth optima at these low temperatures, they were all capable of growth at 5°C, so it is possible that these species could be active under *in-situ* conditions. Interestingly, none of the closest cultured relatives of the isolates were characterized in the literature as being able to grow

below 10°C, it is therefore possible that the isolates described here have adapted to the *in-situ* conditions at the seamounts.

The results of the NaCl tolerance test indicate that the *Halomonas* species is broadly tolerant, while the *Demequina* species is more sensitive to increased NaCl; interestingly, the two strains of *Marinobacter alkaliphilus* exhibited different tolerance profiles. *Halomonas johnsoniae* str T68687, the closest cultured relative of *Halomonas johnsoniae* str KM073, can tolerate between 0 and 20% NaCl and has an optimum of 2.5%; these numbers are reflected in the results for *Halomonas johnsoniae* str KM073 (Barberán et al. 2017). *Demequina salsinemoris* str NBRC 105323 can only tolerate an NaCl concentration up to 8%, while *Demequina salsinemoris* str KM012 can tolerate up to 15% NaCl before growth is halted (Matsumoto et al. 2010). This increased NaCl tolerance could be an adaptation to the environment that these microbes are subjected to at the serpentinite seamounts, additional experiments and genetic analyses are needed to determine the cause for these differences. *Marinobacter alkaliphilus* str ODP1200D-1.5, isolated from South Chamorro Seamount, is able to tolerate a similar NaCl range to *Marinobacter alkaliphilus* str KM012, isolated from Asùt Tesoru Seamount, but does significantly better at concentrations up to 15%. The two seamounts have similar pore water chemistry, with higher NaCl concentrations than the majority of the other seamounts, notably, Asùt Tesoru has a higher concentration of Na^+ (Fryer et al. 2018, Fryer and Salisbury 2006).

The sodium requirement test indicated that all except one of the isolates included in the experiment required sodium to grow at a high pH (pH 10), whereas growth, although reduced, occurred without sodium at a more neutral pH (pH 7). The significantly reduced growth at pH 10 in no sodium media can be attributed to the crucial role that sodium plays in many adaptations

that alkaliphiles employ at high pH, such as pH homeostasis (Padan et al. 2005). However, *Halomonas stevensii* str KM051 did not exhibit reduced growth in the no sodium pH 10 medium, and in fact grew better at high pH than at pH 7. The mechanism for these growth properties is not known.

Table 3. Summary of Results for all Isolates. Dashes indicate that data was not collected for a given isolate.

Isolate	pressure range for growth (MPa)	optimum pressure (MPa)	pH range for growth	optimum pH	temperature range for growth (°C)	optimum temperature (°C)	NaCl concentration range for growth (%)	optimum NaCl concentration (%)	Na required for growth at pH 10 (yes/no)
H. axialensis str KM004	0.1 - 40	0.1	<7 - 10.5	8.5	5 - 47	37	-	-	yes
H. ventosae str KM024	0.1	0.1	<7 - 10	8.5	5 - 47	42	-	-	yes
H. hamiltonii str KM007	0.1 - 40	0.1	<7 - 10	7.5	5 - 47	42	-	-	yes
H. hamiltonii str KM072	0.1 - 30	0.1	<7 - 11	9.0	5 - 47	42	-	-	yes
H. stevensii str KM051	0.1 - 40	0.1	<7 - 10.5	8.5	5 - 47	42	-	-	no
H. johnsoniae str KM073	0.1 - 40	0.1	<7 - 11	9.0	5 - 47	42	0 - 15	0	yes
H. shengliensis str KM026	0.1	0.1	<7 - 10.5	8.0	5 - 47	42	-	-	yes
D. salsinemoris str KM012	0.1 - 40	0.1	<7 - 8.5	7.5	17 - 47	37	0 - 5	0	yes
M. alkaliphilus str KM021	0.1 - 40	0.1	<7 - 10	9.0	5 - 47	42	0 - 15	0	yes
M. alkaliphilus str ODP1200-1.5	0.1 - 30	0.1	<7 - 10	8.5	5 - 47	37	0 - 15	0	yes
M. hydrocarbonoclasticus str KM034	0.1 - 30	0.1	<7 - 9.5	7.0	17 - 47	37	-	-	yes

Genomic Analyses

The four strains that were analyzed, *Halomonas johnsoniae* str KM073, *Halomonas stevensii* str KM051, *Marinobacter alkaliphilus* str KM021, and *Marinobacter alkaliphilus* str ODP1200D-1.5, share many of the same genomic features that could contribute to their ability to inhabit the serpentinite seamounts. Propionate metabolism genes, methane metabolism genes, and the enzyme formate dehydrogenase o were present in all four isolates which suggests that they are able to take advantage of some of the most abundant byproducts of serpentinization (Eickenbusch et al. 2019, Schrenk et al. 2013). Formate dehydrogenase o is primarily expressed under aerobic conditions and liberates electrons by oxidizing formate to carbon dioxide; it has also been implicated in nitrate reduction (Abaibou et al. 1995). In order for the environmental acetate at the seamounts to be taken advantage of, it must first be converted to acetyl-coA which requires the enzyme acetyl-coA synthetase, which all four isolates possess (Cozzone 1998).

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Acetyl-CoA can then be used via the glyoxylate bypass, a series of six reactions, to produce glucose. While all four isolates have the components of the glyoxylate bypass, *Halomonas stevensii* str KM051 and *Marinobacter alkaliphilus* str ODP1200D-1.5 possess additional copies of some of the genes, suggesting that they might be taking advantage of acetate more effectively. The isolates also share several alkaliphilic adaptations, with many of the same Na^+-dependent transporters and at least one Mrp system. The Na^+-antiporter function of the Mrp system plays the crucial role of importing H^+ ions into the cells while extruding Na^+ ions to regulate the intracellular pH (Krulwich et al. 2007). These extruded Na^+ ions can then be used by the Na^+-dependent symporters to import molecules and solutes such as serine, alanine, and pantothenate, a few of the sodium-dependent symporters that are present in the four isolate genomes. In addition to the Mrp system, *Halomonas stevensii* str KM051 and *Marinobacter alkaliphilus* str ODP1200D-1.5 possess NhaD-type Na^+/H^+ antiporters. These antiporters have been shown to have improved function at alkaline pH and have been linked to several alkaliphiles (Kurz et al. 2006, Liu et al. 2005, Nozaki et al. 1998, Williamson et al. 2016). The differences in the growth rates during the pH characterizations of the two *Marinobacter alkaliphilus* strains could be explained by the fact that the genome of strain ODP1200D-1.5 was found to encode two of these NhaD-type antiporters, while strain KM021 did not encode any NhaD-type antiporters.

The predicted proteins of all of the isolates were compared with the predicted proteins of closely related species. Most of the proteins in the *Halomonas stevensii* str KM051 genome were approximately 95% identical with the proteins encoded by the genome of *Halomonas stevensii* str S18214, and approximately 80% identical with the proteins encoded by the draft genome of *Halomonas alkaliphila* LS44. The genomes of the two *Halomonas stevensii* strains are very similar, encoding many of the same pathways. Some key differences between the two strains

include an oxaloacetate decarboxylase, cbb3-type cytochrome, and an arsenic resistance mechanism that are all encoded in the genome of strain KM051 and not in the genome of strain S18214. The Na^+/H^+ antiporter function of the oxaloacetate decarboxylase could confer higher pH tolerance to strain KM051, while the cbb3-type cytochrome has been implicated in adaptation to increased pressure (Chikuma et al. 2007, Dibrov et al. 1986, Dimroth 1982, Lietzan and Maurice 2014, Liu et al. 2020, Simonato et al. 2006). Serpentinization has been linked to elevated concentrations of arsenic, and the presence of this resistance mechanism in *Halomonas stevensii* str KM051 could be an adaptation to the environment on the seamounts (Ryan et al. 2019).

The proteome of *Halomonas johnsoniae* str KM073 exhibited much larger differences with the proteomes of all three of the above strains, with most of the proteins encoded by its genome being between 30% and 70% identical to the proteins encoded by the genomes of *Halomonas alkaliphila* LS44, *Halomonas stevensii* str S18214, and *Halomonas stevensii* str KM051. However, *Halomonas johnsoniae* str KM073 and *Halomonas stevensii* str KM051 do share many of the same metabolic capabilities and pathways. The major differences between the two species include an acyclic terpene utilization pathway and a few additional aromatic compound utilization pathways encoded by the *Halomonas johnsoniae* str KM073 genome, and the NhaD-type transporters encoded by the *Halomonas stevensii* str KM051 genome. There were no obvious genetic differences identified that would explain the growth of *Halomonas stevensii* str KM051 at elevated pH in the no sodium media; further physical and genomic tests are required to explain this phenomenon.

Most of the proteins encoded by the genome of *Marinobacter alkaliphilus* str ODP1200D-1.5 are approximately 95% identical to the proteins encoded by the genome of the

closely related species *Marinobacter shengliensis* str SLO13A34A2, and between 30% and 80% identical to the proteins encoded by *Marinobacter alkaliphilus* str KM021. The proteome of *Marinobacter alkaliphilus* str KM021 suggest that it is capable of ammonification, denitrification, and acyclic terpene utilization while that of strain ODP1200D-1.5 does not show evidence of these pathways. *Marinobacter alkaliphilus* str ODP1200D-1.5 encodes NhaD-type antiporters and the enzyme urease, neither of which are evident in strain KM021. Beyond these differences, the two strains encode many of the same metabolic capabilities and pathways.

Future Directions

It would be interesting to determine if any of the isolates in this study could have reached the seamounts through subduction and reemergence, as opposed to having repopulated the serpentinizing fluids as they rose through the Philippine plate or settled out of the water column. This could be evaluated by performing pressure recovery tests, incubating the isolates at pressures up to 400MPa for up to several months and then transferring the incubations to reduced and atmospheric pressure incubations. This experiment could also be combined with increased temperatures between 80°C and 350°C for the long-term incubations to further mimic the conditions that a bacterial community subducted on the top of a seamount would experience in the subduction channel.

Two of the isolates, *Halomonas ventosae* str KM024 and *Halomonas shengliensis* str KM026, showed little to no growth at all conditions in the pressure tests. I believe that this was because they were incubated in the bulbs in their isolation medium, Alkaline MJYTGL Medium pH 10.5. There is little opportunity for air exchange under these conditions, so it is likely that the medium remained at pH 10.5 for the duration of the experiment, whereas they were isolated on agar plates that were not pH checked before pouring. Autoclaving the MJYTGL medium

significantly lowered the pH in all cases, and without subsequent adjustment the pH of the

MJYTGL 1.7% agar plates would most likely be between pH 9 and 10. The fact that they were

isolated on MJYTGL agar that had been adjusted to a pH of 10.5 before autoclaving does not

indicate that they can grow in liquid media at a pH maintained at 10.5. These two isolates should

be tested again for pressure tolerance in a lower pH medium, such as Alkaline MJYTGL

Medium pH 9, since they did not show robust growth above pH 10 in the pH tests, which were

conducted after the pressure tests.

When possible, it will be important to obtain data on the microbial communities present

in the sediment samples. While DNA extraction from the sediments has not been successful thus

far, further avenues need to be explored. RNA extraction from amended sediments has been

attempted with minimal success, preliminary data was obtained with very low sequence depth.

Strategies that have yet to be tested include density-gradient centrifugation to separate the

microbial cells from the sediment particles prior to DNA extraction (Kallmeyer et al. 2008).

While it would not give a complete picture of the community, it might also be valuable to

directly incubate small aliquots of sediment in liquid media instead of plating the sediments on

solid agar media. Illumina tag sequencing of the liquid cultures might reveal different species

enrichments from those on plates.

Most probable number estimations of sediment enrichments could also be performed at a

variety of pHs to determine the fraction of the culturable community that is alkali-sensitive,

alkalitolerant, or alkaliphilic. The serpentinite seamounts are predicted to harbor communities

dominated by, but not exclusive to, alkaliphiles (Fryer et al. 2020). The composition of cultures

grown at close to *in-situ* pressure and temperature, as well as in a variety of other combinations,

could provide interesting insights into the physical-chemical adaptations of the culturable

communities at the seamounts and might provide hints as to which microbes are simply present in samples and which might actually be active *in situ*.

If possible, pH studies in a chemostat where pH can be continuously maintained and exponential growth can be measured stably would be valuable for ensuring the accuracy of the experiments and to increase the precision of the values for maximum and optimum pH.

It would be valuable to expand these characterization tests to more of the isolates obtained in the initial plating in order to get a better picture of what the culturable microbes in these samples are capable of tolerating. The only species that was isolated from the plates that was not fully characterized is *Halomonas alkalicola;* this isolate was identified later and was subjected to pH experiments with unexceptional results. However, the differences between the two *Halomonas hamiltonii* strains in this study indicate that investigating duplicates of the same species may be interesting when they are isolated from different samples. It would also be interesting to re-plate the sediments at conditions that have been determined to be optimal for many of the ten isolates examined in this study and see if greater diversity is obtained or if the known isolates dominate.

REFERENCES

1. Abaibou, H., Pommier, J., Benoit, S., Giordano, G., & Mandrand-Berthelot, M. A. (1995). Expression and characterization of the Escherichia coli fdo locus and a possible physiological role for aerobic formate dehydrogenase. *Journal of bacteriology*, *177*(24), 7141-7149.

2. Aziz, R. K., Bartels, D., Best, A. A., Dejongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E. M., Kubal, M., Meyer, F., Olsen, G. J., Olson, R., Osterman, A. L., Overbeek, R. A., McNeil, L. K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G. D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., & Zagnitko, O. (2008). The RAST Server: Rapid Annotations using Subsystems Technology. *BMC Genomics*, *9*(1), 75.

3. Bada, J. L. (2002). Origin of Life: Some Like It Hot, But Not the First Biomolecules. *Science*, *296*(5575), 1982-1983.

4. Barberán, A., Caceres Velazquez, H., Jones, S., & Fierer, N. (2017). Hiding in Plain Sight: Mining Bacterial Species Records for Phenotypic Trait Information. *mSphere*, *2*(4), e00237-00217.

5. Barge, L. M., Branscomb, E., Brucato, J. R., Cardoso, S. S. S., Cartwright, J. H. E., Danielache, S. O., Galante, D., Kee, T. P., Miguel, Y., Mojzsis, S., Robinson, K. J., Russell, M. J., Simoncini, E., & Sobron, P. (2017). Thermodynamics, Disequilibrium, Evolution: Far-From-Equilibrium Geological and Chemical Considerations for Origin-Of-Life Research. *Origins of Life and Evolution of Biospheres*, *47*(1), 39-56.

6. Brazelton, W. J., Ludwig, K. A., Sogin, M. L., Andreishcheva, E. N., Kelley, D. S., Shen, C.-C., Edwards, R. L., & Baross, J. A. (2010). Archaea and bacteria with surprising microdiversity show shifts in dominance over 1,000-year time scales in hydrothermal chimneys. *Proceedings of the National Academy of Sciences*, *107*(4), 1612-1617.

7. Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., Olson, R., Overbeek, R., Parrello, B., Pusch, G. D., Shukla, M., Thomason, J. A., Stevens, R., Vonstein, V., Wattam, A. R., & Xia, F. (2015). RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific Reports*, *5*(1), 8365.

8. Brito, E. M. S., Guyoneaud, R., Goñi-Urriza, M., Ranchou-Peyruse, A., Verbaere, A., Crapez, M. A. C., Wasserman, J. C. A., & Duran, R. (2006). Characterization of hydrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil. *Research in Microbiology*, *157*(8), 752-762.

9. Cheftel, J. C. (1995). Review: High-pressure, microbial inactivation and food preservation. *Food Science and Technology International*, *1*(2-3), 75-90.

10. Chikuma, S., Kasahara, R., Kato, C., & Tamegai, H. (2007). Bacterial adaptation to high pressure: a respiratory system in the deep-sea bacterium Shewanella violaceaDSS12. *FEMS Microbiology Letters*, *267*(1), 108-112.

11. Cozzone, A. J. (1998). Regulation of Acetate Metabolism by Protein Phosphorylation in Enteric Bacteria. *Annual Review of Microbiology*, *52*(1), 127-164.

12. Curtis, A. C., Wheat, C. G., Fryer, P., & Moyer, C. L. (2013). Mariana Forearc Serpentinite Mud Volcanoes Harbor Novel Communities of Extremophilic Archaea. *Geomicrobiology Journal*, *30*(5), 430-441.

13. Czech, L., Hermann, L., Stöveken, N., Richter, A. A., Höppner, A., Smits, S. H. J., Heider, J., & Bremer, E. (2018). Role of the Extremolytes Ectoine and Hydroxyectoine as Stress Protectants and Nutrients: Genetics, Phylogenomics, Biochemistry, and Structural Analysis. *Genes*, *9*(4), 177.

14. De Mets, F., Van Melderen, L., & Gottesman, S. (2019). Regulation of acetate metabolism and coordination with the TCA cycle via a processed small RNA. *Proceedings of the National Academy of Sciences*, *116*(3), 1043-1052.

15. Deamer, D., Singaram, S., Rajamani, S., Kompanichenko, V., & Guggenheim, S. (2006). Self-assembly processes in the prebiotic environment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1474), 1809-1818.

16. Dibrov, P. A., Kostyrko, V. A., Lazarova, R. L., Skulachev, V. P., & Smirnova, I. A. (1986). The sodium cycle. I. Na+-dependent motility and modes of membrane energization in the marine alkalotolerant Vibrio alginolyticus. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *850*(3), 449-457.

17. Dimroth, P. (1982). The generation of an electrochemical gradient of sodium ions upon decarboxylation of oxaloacetate by the membrane-bound and Na+-activated oxaloacetate decarboxylase from Klebsiella aerogenes. *Eur J Biochem*, *121*(2), 443-449.

18. Eickenbusch, P., Takai, K., Sissman, O., Suzuki, S., Menzies, C., Sakai, S., Sansjofre, P., Tasumi, E., Bernasconi, S. M., Glombitza, C., Jørgensen, B. B., Morono, Y., & Lever, M. A. (2019). Origin of Short-Chain Organic Acids in Serpentinite Mud Volcanoes of the Mariana Convergent Margin. *Frontiers in Microbiology*, *10*, 1729.

19. Fryer, P. (1996). Evolution of the Mariana Convergent Plate Margin System. *Reviews of Geophysics*, *34*(1), 89-125.

20. Fryer, P. (2012). Serpentinite Mud Volcanism: Observations, Processes, and Implications. *Annual Review of Marine Science*, *4*(1), 345-373.

21. Fryer, P., & Mottl, M. J. (1997). *Shinkai 6500* investigations of a resurgent mud volcano on the southeastern Mariana forearc. *JAMSTEC Journal of Deep Sea Research*, *13*, 103-114.

22. Fryer, P., Saboda, C. L., Johnson, L. E., Mackay, M. E., Moore, G. F., & Stoffers, P. (1990). Conical Seamount: seaMARC II, *Alvin* Submersible, and Seismic Reflection Studies. *Proceedings of the Ocean Drilling Program, Initial Reports*, *125*, 69-80.

23. Fryer, P., & Salisbury, M. H. (2006). Leg 195 Synthesis; Site 1200; serpentinite seamounts of the Izu-Bonin/Mariana convergent plate margin, ODP Leg 125 and 195 drilling results. *Proceedings of the ocean drilling program, scientific results*, *195*, 30.

24. Fryer, P., Wheat, C. G., Williams, T., Albers, E., Bekins, B., Debret, B. P. R., Deng, R. J., Dong, Y., Eickenbusch, P., Frery, E. A., Ichiyama, Y., Johnson, K., Johnston, R. M., Kevorkian, R. T., Kurz, W., Magalhaes, V., Mantovanelii, S. S., Menapace, W., Menzies, C. D., Michibayashi, K., Moyer, C. L., Mullane, K. K., Park, J.-W., Price, R. E., Ryan, J. G., Shervais, J. W., Sissman, O. J., Suzuki, S., Takai, K., Walter, B., & Zhang, R. (2018a). Expedition 366 methods. *Proceedings of the International Ocean Discovery Program*, *366*, 24.

25. Fryer, P., Wheat, C. G., Williams, T., Albers, E., Bekins, B., Debret, B. P. R., Deng, R. J., Dong, Y., Eickenbusch, P., Frery, E. A., Ichiyama, Y., Johnson, K., Johnston, R. M., Kevorkian, R. T., Kurz, W., Magalhaes, V., Mantovanelii, S. S., Menapace, W., Menzies, C. D., Michibayashi, K., Moyer, C. L., Mullane, K. K., Park, J.-W., Price, R. E., Ryan, J. G., Shervais, J. W., Sissman, O. J., Suzuki, S., Takai, K., Walter, B., & Zhang, R. (2018b). Expedition 366 Summary. *Proceedings of the International Ocean Discovery Program*, *366*, 1-23.

26. Fryer, P., Wheat, C. G., Williams, T., Kelley, C., Johnson, K., Ryan, J., Kurz, W., Shervais, J., Albers, E., Bekins, B., Debret, B., Deng, J., Dong, Y., Eickenbusch, P., Frery, E., Ichiyama, Y., Johnston, R., Kevorkian, R., Magalhaes, V., Mantovanelli, S., Menapace, W., Menzies, C., Michibayashi, K., Moyer, C., Mullane, K., Park, J.-W., Price, R., Sissmann, O., Suzuki, S., Takai, K., Walter, B., Zhang, R., Amon, D., Glickson, D., & Pomponi, S. (2020). Mariana serpentinite mud volcanism exhumes subducted seamount materials: implications for the origin of life. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *378*(2165), 20180425.

27. Fujisawa, M., Fackelmayer, O. J., Liu, J., Krulwich, T. A., & Hicks, D. B. (2010). The ATP Synthase A-Subunit of Extreme Alkaliphiles is a Distinct Variant: Mutations in the Critical Alkaliphile-Specific Residue Lys-180 and Other Residues that Support

Alkaliphile Oxidative Phosphorylation. *American Society for Biochemistry & Molecular Biology*, *285*(42), 32105-32115.

28. Gauthier, M. J., Lafay, B., Christen, R., Fernandez, L., Acquaviva, M., Bonin, P., & Bertrand, J. C. (1992). Marinobacter hydrocarbonoclasticus gen. nov., sp. nov., a New, Extremely Halotolerant, Hydrocarbon-Degrading Marine Bacterium. *International Journal of Systematic Bacteriology*, *42*(4), 568-576.

29. Glein, C. R., Baross, J. A., & Waite, J. H. (2015). The pH of Enceladus' ocean. *Geochimica et Cosmochimica Acta*, *162*, 202-219.

30. Gordon, A. L. (2001). Bottom water formation. In *Encyclopedia of Ocean Sciences (Third Edition)* (pp. 120-126).

31. Guffanti, A. A., & Krulwich, T. A. (1992). Features of Apparent Nonchemiosmotic Energization of Oxidative-Phosphorylation by Alkaliphilic Bacillus-Firmus OF4. *Journal of Biological Chemistry*, *267*(14), 9580-9588.

32. Haggerty, J. A., & Fisher, J. B. (1992). Short-Chain Organic Acids in Interstitial Waters from Mariana and Bonin Forearc Serpentines: Leg 125. *Proceedings of the ocean drilling program, scientific results*, *125*, 387–395.

33. Halevy, I., & Bachan, A. (2017). The geologic history of seawater pH. *Science*, *355*(6329), 1069-1071.

34. Hamada, M., Tamura, T., Yamamura, H., Suzuki, K. I., & Hayakawa, M. (2013). Demequina flava sp. nov. and Demequina sediminicola sp. nov., isolated from sea sediment. *63*, 249-253.

35. Hastie, A. R., Fitton, J. G., Bromiley, G. D., Butler, I. B., & Odling, N. W. A. (2016). The origin of Earth's first continents and the onset of plate tectonics. *Geology*, *44*(10), 855-858.

36. Hess, H. H. (1955). Serpentinites, orogeny, and epeirogeny. *Geological Society of America Special Paper*, *62*, 391–408.

37. Hicks, D. B., & Krulwich, T. A. (1995). The respiratory chain of alkaliphilic bacteria. *Biochimica et Biophysica Acta - Bioenergetics*, *1229*(3), 303-314.

38. Hiramatsu, T., Kodama, K., Kuroda, T., Mizushima, T., & Tsuchiya, T. (1998). A putative multisubunit Na+/H+ antiporter from Staphylococcus aureus. *Journal of bacteriology*, *180*(24), 6642-6648.

39. Horikoshi, K. (1971). Production of Alkaline Enzymes by Alkalophilic Microorganisms. *Agricultural and Biological Chemistry*, *35*(11), 1783-1791.

40. Horikoshi, K. (1999). Alkaliphiles: Some Applications of their Products for Biotechnology. *Microbiology and Molecular Biology Reviews*, *63*(4), 735-750.

41. Horikoshi, K. (2004). Alkaliphiles. *Proceedings of the Japan Academy, Series B*, *80*, 166-178.

42. Horikoshi, K., & Akiba, T. (1982). *Alkalophilic microorganisms. A new microbial world* (Vol. 5). Springer Verlag KG.

43. Hulme, S. M., Wheat, C. G., Fryer, P., & Mottl, M. J. (2010). Pore water chemistry of the Mariana serpentinite mud volcanoes: A window to the seismogenic zone. *Geochemistry, Geophysics, Geosystems*, *11*(1), Q01X09.

44. Huu, N. B., Denner, E. B. M., Ha, D. T. C., Wanner, G., & Stan-Lotter, H. (1999). Marinobacter aquaeolei sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well. *International Journal of Systematic and Evolutionary Microbiology*, *49*(2), 367-375.

45. Kallmeyer, J., Smith, D. C., Spivack, A. J., & D'Hondt, S. (2008). New cell extraction procedure applied to deep subsurface sediments. *Limnology and Oceanography: Methods*, *6*(6), 236-245.

46. Kashefi, K. (2003). Extending the Upper Temperature Limit for Life. *Science*, *301*(5635), 934-934.

47. Kaye, J. Z., Márquez, M. C., Ventosa, A., & Baross, J. A. (2004). Halomonas neptunia sp. nov., Halomonas sulfidaeris sp. nov., Halomonas axialensis sp. nov. and Halomonas hydrothermalis sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. *International Journal of Systematic and Evolutionary Microbiology*, *54*(2), 499-511.

48. Kim, J.-O., Lee, H.-J., Han, S.-I., & Whang, K.-S. (2017). Marinobacter halotolerans sp. nov., a halophilic bacterium isolated from a saltern crystallizing pond. *International Journal of Systematic and Evolutionary Microbiology*, *67*(2), 460-465.

49. Kim, K. K., Lee, K. C., Jeong, H., Stevens, D. A., & Lee, J.-S. (2012). Draft Genome Sequence of the Human Pathogen Halomonas stevensii S18214T. *Journal of Bacteriology*, *194*(18), 5143-5143.

50. Kim, K. K., Lee, K. C., Oh, H.-M., & Lee, J.-S. (2010). Halomonas stevensii sp. nov., Halomonas hamiltonii sp. nov. and Halomonas johnsoniae sp. nov., isolated from a renal care centre. *International Journal of Systematic and Evolutionary Microbiology*, *60*(2), 369-377.

51. Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*(2), 111-120.

52. Kitada, M., Kosono, S., & Kudo, T. (2000). The Na + /H + antiporter of alkaliphilic Bacillus sp. *Extremophiles*, *4*(5), 253-258.

53. Krulwich, T. A., Liu, J., Morino, M., Fujisawa, M., Ito, M., & Hicks, D. B. (2011). Adaptive Mechanisms of Extreme Alkaliphiles. In *Extremophiles Handbook* (pp. 119-139). Springer Japan.

54. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, *35*(6), 1547-1549.

55. Kurz, M., Brünig, A. N., & Galinski, E. A. (2006). NhaD type sodium/proton-antiporter of Halomonas elongata: a salt stress response mechanism in marine habitats? *Saline Systems*, *2*, 10.

56. Lane, N., Allen, J. F., & Martin, W. (2010). How did LUCA make a living? Chemiosmosis in the origin of life. *BioEssays*, *32*(4), 271-280.

57. Letunic, I., & Bork, P. (2019). Interactive Tree of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research*, *47*(W1), W256-W259.

58. Lietzan, A. D., & Maurice, M. S. (2014). Functionally diverse biotin-dependent enzymes with oxaloacetate decarboxylase activity. *Archives of Biochemistry and Biophysics*, *544*, 75-86.

59. Liu, J., Xue, Y., Wang, Q., Wei, Y., Swartz, T. H., Hicks, D. B., Ito, M., Ma, Y., & Krulwich, T. A. (2005). The Activity Profile of the NhaD-Type Na+(Li+)/H+ Antiporter from the Soda Lake Haloalkaliphile Alkalimonas amylolytica Is Adaptive for the Extreme Environment. *Journal of Bacteriology*, *187*(22), 7589-7595.

60. Liu, P., Ding, W., Lai, Q., Liu, R., Wei, Y., Wang, L., Xie, Z., Cao, J., & Fang, J. (2020). Physiological and genomic features of Paraoceanicella profunda gen. nov., sp. nov., a novel piezophile isolated from deep seawater of the Mariana Trench. *Microbiology Open*, *9*(2), e966.

61. Ludwig, K. A., Kelley, D. S., Butterfield, D. A., Nelson, B. K., & Früh-Green, G. (2006). Formation and evolution of carbonate chimneys at the Lost City Hydrothermal Field. *Geochimica et Cosmochimica Acta*, *70*(14), 3625-3645.

62. Martínez-Cánovas, M. J., Quesada, E., Llamas, I., & Béjar, V. (2004). Halomonas ventosae sp. nov., a moderately halophilic, denitrifying, exopolysaccharide-producing

bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *54*(3), 733-737.

63. Matsumoto, A., Nakai, K., Morisaki, K., Ōmura, S., & Takahashi, Y. (2010). Demequina salsinemoris sp. nov., isolated on agar media supplemented with ascorbic acid or rutin. *International Journal of Systematic and Evolutionary Microbiology*, *60*(5), 1206-1209.

64. Matsuno, T., Goto, T., Ogami, S., Morimoto, H., Yamazaki, K., Inoue, N., Matsuyama, H., Yoshimune, K., & Yumoto, I. (2018). Formation of Proton Motive Force Under Low-Aeration Alkaline Conditions in Alkaliphilic Bacteria. *Frontiers in Microbiology*, *9*, 2331.

65. McCollom, T. M., & Seewald, J. S. (2013). Serpentinites, Hydrogen, and Life. *Elements*, *9*(2), 129-134.

66. Mottl, M. J., Komor, S. C., Fryer, P., & Moyer, C. L. (2003). Deep-slab fluids fuel extremophilic Archaeaon a Mariana forearc serpentinite mud volcano: Ocean Drilling Program Leg 195. *Geochemistry, Geophysics, Geosystems*, *4*(9009), 11.

67. Nozaki, K., Kuroda, T., Mizushima, T., & Tsuchiya, T. (1998). A new Na+/H+ antiporter, NhaD, of Vibrio parahaemolyticus. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1369*(2), 213-220.

68. O'Reilly, C. E., O'Connor, P. M., Kelly, A. L., Beresford, T. P., & Murphy, P. M. (2000). Use of Hydrostatic Pressure for Inactivation of Microbial Contaminants in Cheese. *Applied and Environmental Microbiology*, *66*(11), 4890-4896.

69. Oakley, A. J., Taylor, B., Fryer, P., Moore, G. F., Goodliffe, A. M., & Morgan, J. K. (2007). Emplacement, growth, and gravitational deformation of serpentinite seamounts on the Mariana forearc. *Geophysical Journal International*, *170*(2), 615-634.

70. Padan, E., Bibi, E., Ito, M., & Krulwich, T. A. (2005). Alkaline pH homeostasis in bacteria: New insights. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1717*(2), 67-88.

71. Park, S., Won, S.-M., Lee, J.-S., Jung, Y.-T., & Yoon, J.-H. (2015). Demequina activiva sp. nov., isolated from a tidal flat. *65*(7), 2042-2047.

72. Peresypkin, V. V., Lein, A. Y., Bodganov, Y. A., & Bortnikov, N. S. (1999). On the nature of lipids in hydrothermal formations at the Broken Spur and the vent field of the Mid-Atlantic Ridge. *Exploration and Mining Geology*, *8*(3-4), 365-377.

73. Plümper, O., King, H. E., Geisler, T., Liu, Y., Pabst, S., Savov, I. P., Rost, D., & Zack, T. (2017). Subduction zone forearc serpentinites as incubators for deep microbial life. *Proceedings of the National Academy of Sciences*, *114*(17), 4324-4329.

74. Pons, M. L., Quitte, G., Fujii, T., Rosing, M. T., Reynard, B., Moynier, F., Douchet, C., & Albarede, F. (2011). Early Archean serpentine mud volcanoes at Isua, Greenland, as a niche for early life. *Proceedings of the National Academy of Sciences*, *108*(43), 17639-17643.

75. Preiner, M., Xavier, J., Sousa, F., Zimorski, V., Neubeck, A., Lang, S., Greenwell, H., Kleinermanns, K., Tüysüz, H., McCollom, T., Holm, N., & Martin, W. (2018). Serpentinization: Connecting Geochemistry, Ancient Metabolism and Industrial Hydrogenation. *Life*, *8*(4), 41.

76. Preiss, L., Hicks, D. B., Suzuki, S., Meier, T., & Krulwich, T. A. (2015). Alkaliphilic Bacteria with Impact on Industrial Applications, Concepts of Early Life Forms, and Bioenergetics of ATP Synthesis. *Frontiers in Bioengineering and Biotechnology*, *3*, 75.

77. Russell, M. J., Hall, A. J., & Martin, W. (2010). Serpentinization as a source of energy at the origin of life. *Geobiology*, *8*(5), 355-371.

78. Ryan, P. C., Huertas, F. J., Pincus, L. N., & Painter, W. (2019). Arsenic-Bearing Serpentine-Group Minerals: Mineral Synthesis with Insights for the Arsenic Cycle. *Clays and Clay Minerals*, *67*(6), 488-506.

79. Sako, Y., Takai, K., Ishida, Y., Uchida, A., & Katayama, Y. (1996). Rhodothermus obamensis sp. nov., a Modern Lineage of Extremely Thermophilic Marine Bacteria. *International Journal of Systematic Bacteriology*, *46*(4), 1099-1104.

80. Salisbury, M. H., Shinohara, M., Richter, C., Araki, E., Barr, S. R., D'Antonio, M., Dean, S. M., Diekmann, B., Edwards, K. M., Fryer, P. B., Gaillot, P. J., Hammon, W. S. I., Hart, D., Januszczak, N., Komor, S. C., Kristensen, M. B., Lockwood, J. P., Mottl, M. J., Moyer, C. L., Nakahigashi, K., Savov, I. P., Su, X., Wei, K.-Y., & Yamada, T. (2002). Site 1200. *Proceedings of the ocean drilling program, initial reports, Leg 195: College Station, TX (Ocean Drilling Program)*, 1-173.

81. Schiffman, P., Zierenberg, R., Chadwick, W. W., Clague, D. A., & Lowenstern, J. (2010). Contamination of basaltic lava by seawater: Evidence found in a lava pillar from Axial Seamount, Juan de Fuca Ridge. *Geochemistry, Geophysics, Geosystems*, *11*(4), Q04004.

82. Schrenk, M. O., Brazelton, W. J., & Lang, S. Q. (2013). Serpentinization, Carbon, and Deep Life. *Reviews in Mineralogy and Geochemistry*, *75*(1), 575-606.

83. Schulte, M., Blake, D., Hoehler, T., & McCollom, T. (2006). Serpentinization and Its Implications for Life on the Early Earth and Mars. *Astrobiology*, *6*(2), 364-376.

84. Servin, J. A., Herbold, C. W., Skophammer, R. G., & Lake, J. A. (2007). Evidence Excluding the Root of the Tree of Life from the Actinobacteria. *Molecular Biology and Evolution*, *25*(1), 1-4.

85. Shervais, J. W., Kolesar, P., & Andreasen, K. (2005). A Field and Chemical Study of Serpentinization—Stonyford, California: Chemical Flux and Mass Balance. *International Geology Review*, *47*(1), 1-23.

86. Simonato, F., Campanaro, S., Lauro, F. M., Vezzi, A., D'Angelo, M., Vitulo, N., Valle, G., & Bartlett, D. H. (2006). Piezophilic adaptation: a genomic point of view. *Journal of Biotechnology*, *126*(1), 11-25.

87. Sleep, N., Bird, D., & Pope, E. (2011). Serpentinite and the dawn of life. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *366*(1580), 2857-2869.

88. Suzuki, S., Kuenen, J. G., Schipper, K., Van Der Velde, S., Ishii, S. I., Wu, A., Sorokin, D. Y., Tenney, A., Meng, X., Morrill, P. L., Kamagata, Y., Muyzer, G., & Nealson, K. H. (2014). Physiological and genomic features of highly alkaliphilic hydrogen-utilizing Betaproteobacteria from a continental serpentinizing site. *Nature Communications*, *5*(1), 3900.

89. Swartz, T., Ikewada, S., Ishikawa, O., Ito, M., & Krulwich, T. A. (2005). The Mrp system: a giant among monovalent cation/proton antiporters? *Extremophiles*, *9*(5), 345-354.

90. Takai, K., Moser, D. P., Onstott, T. C., Spoelstra, N., Pfiffner, S. M., Dohnalkova, A., & Fredrickson, J. K. (2001). Alkaliphilus transvaalensis gen. nov., sp. nov., an extremely alkaliphilic bacterium isolated from a deep South African gold mine. *International Journal of Systematic and Evolutionary Microbiology*, *51*(4), 1245-1256.

91. Takai, K., Moyer, C. L., Miyazaki, M., Nogi, Y., Hirayama, H., Nealson, K. H., & Horikoshi, K. (2005). Marinobacter alkaliphilus sp. nov., a novel alkaliphilic bacterium isolated from subseafloor alkaline serpentine mud from Ocean Drilling Program Site 1200 at South Chamorro Seamount, Mariana Forearc. *Extremophiles*, *9*(1), 17-27.

92. Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*(3), 512-516.

93. Tyler, P. A., & Young, C. M. (2003). Dispersal at hydrothermal vents: a summary of recent progress. *Hydrobiologia*, *503*(1-3), 9-19.

94. Ue, H., Matsuo, Y., Kasai, H., & Yokota, A. (2011). Demequina globuliformis sp. nov., Demequina oxidasica sp. nov. and Demequina aurantiaca sp. nov., actinobacteria isolated from marine environments, and proposal of Demequinaceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, *61*(6), 1322-1329.

95. Vreeland, R. H. Halomonas. In *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1-19).

96. Wang, Y.-N., Cai, H., Chi, C.-Q., Lu, A.-H., Lin, X.-G., Jiang, Z.-F., & Wu, X.-L. (2007). Halomonas shengliensis sp. nov., a moderately halophilic, denitrifying, crude-oil-utilizing bacterium. *International Journal of Systematic and Evolutionary Microbiology*, *57*(6), 1222-1226.

97. Wattam, A. R., Davis, J. J., Assaf, R., Boisvert, S., Brettin, T., Bun, C., Conrad, N., Dietrich, E. M., Disz, T., Gabbard, J. L., Gerdes, S., Henry, C. S., Kenyon, R. W., Machi, D., Mao, C., Nordberg, E. K., Olsen, G. J., Murphy-Olson, D. E., Olson, R., Overbeek, R., Parrello, B., Pusch, G. D., Shukla, M., Vonstein, V., Warren, A., Xia, F., Yoo, H., & Stevens, R. L. (2017). Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. *Nucleic Acids Research*, *45*(D1), D535-D542.

98. Watts, A. B., Koppers, A. A. P., & Robinson, D. P. (2010). Seamount Subduction and Earthquakes. *Oceanography*, *23*(1), 166-173.

99. Wernick, D. G., Choi, K.-Y., Tat, C. A., Lafontaine Rivera, J. G., & Liao, J. C. (2013). Genome Sequence of the Extreme Obligate Alkaliphile Bacillus marmarensis Strain DSM 21297. *Genome Announcements*, *1*(6), e00967-00913.

100. Wheat, C., Fryer, P., Fisher, A., Hulme, S., Jannasch, H., Mottl, M., & Becker, K. (2008). Borehole observations of fluid flow from South Chamorro Seamount, an active serpentinite mud volcano in the Mariana forearc. *267*(3-4), 401-409.

101. Wilde, S. A., Valley, J. W., Peck, W. H., & Graham, C. M. (2001). Evidence from detrital zircons for the existence of continental crust and oceans on the Earth 4.4 Gyr ago. *Nature*, *409*(6817), 175-178.

102. Williamson, A., De Santi, C., Altermark, B., Karlsen, C., & Hjerde, E. (2016). Complete genome sequence of Halomonas sp. R5-57. *Standards in Genomic Sciences*, *11*(1), 62.

103. Yayanos, A. A. (2001). Deep-sea piezophilic bacteria. *Methods in Microbiology*, 615-637.

104. Yi, H., Schumann, P., & Chun, J. (2007). Demequina aestuarii gen. nov., sp. nov., a novel actinomycete of the suborder Micrococcineae, and reclassification of Cellulomonas
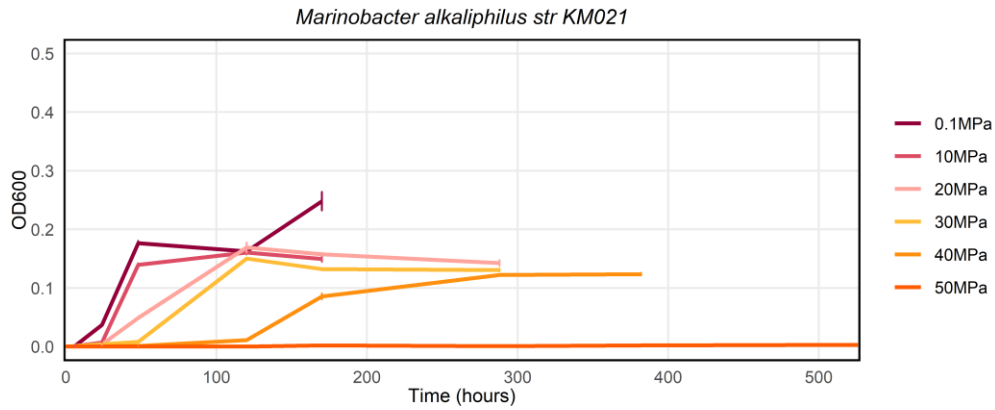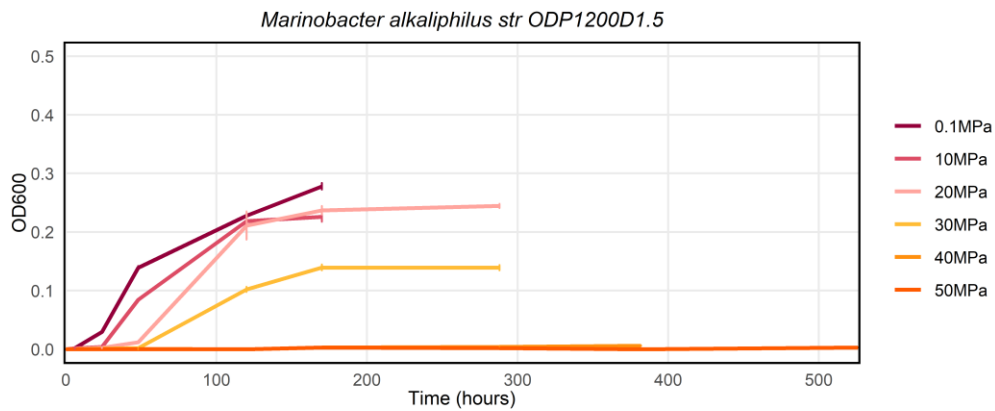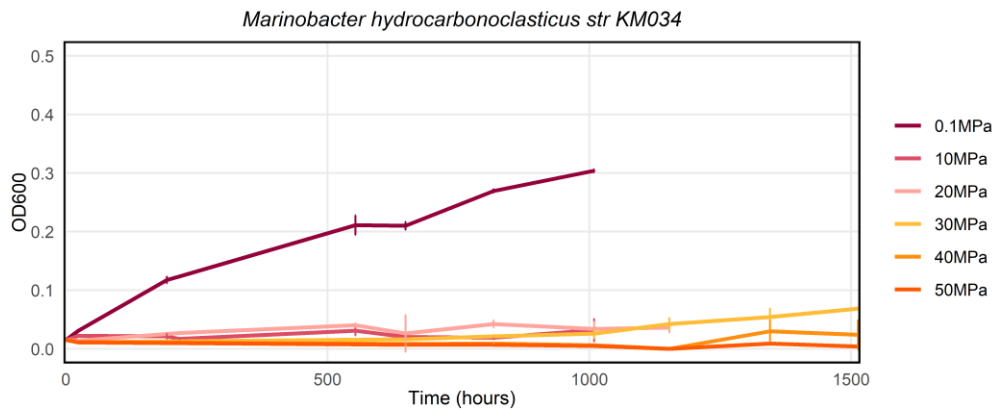
fermentans Bagnara et al. 1985 as Actinotalea fermentans gen. nov., comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *57*(1), 151-156.

105. Zerbino, D. R., & Birney, E. (2008). Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. *Genome Research*, *18*(5), 821-829.

106. Zierenberg, R. A., Adams, M. W. W., & Arp, A. J. (2000). Life in extreme environments: Hydrothermal vents. *Proceedings of the National Academy of Sciences*, *97*(24), 12961-12962
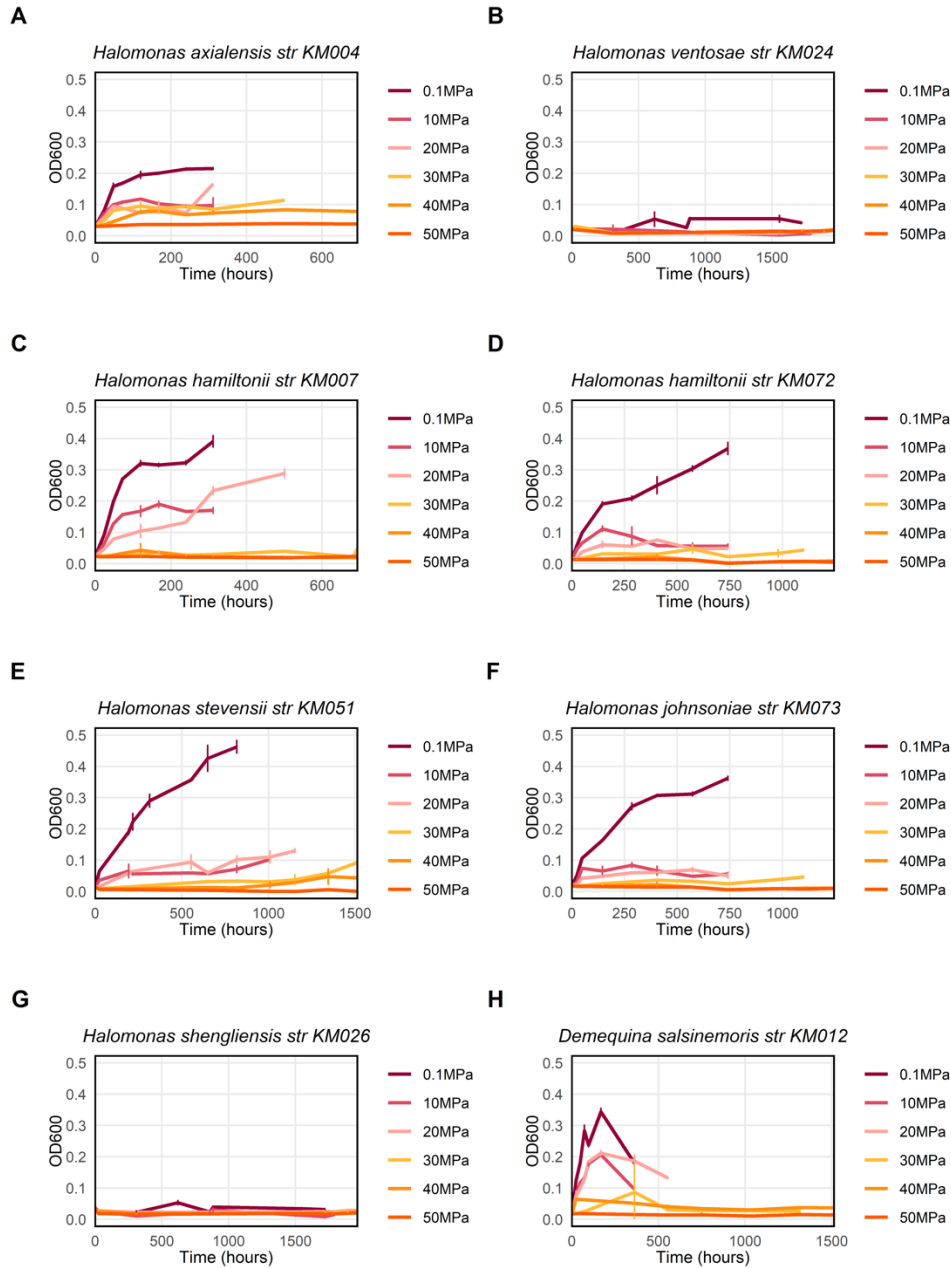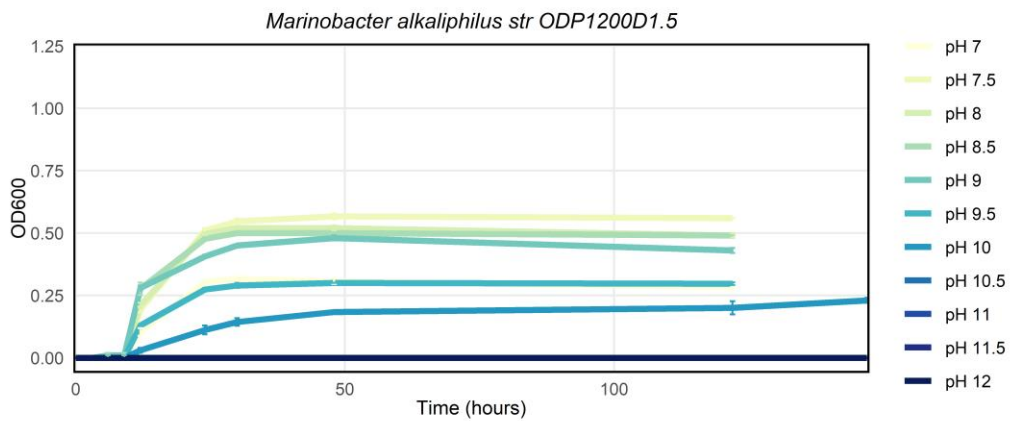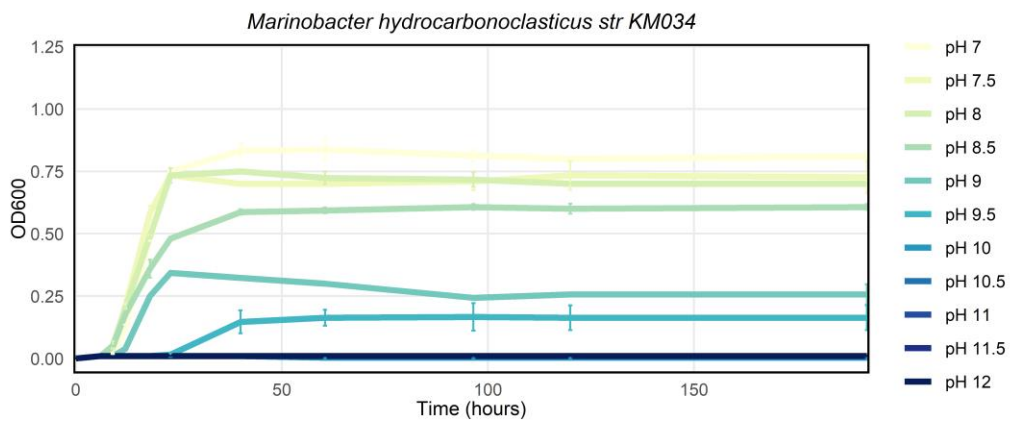
Supplementary Figure 1. Pressure Growth Curves for *Halomonas* and *Demequina* isolates. (A) *Halomonas axialensis* str KM004, incubated in MB2216, (B) *Halomonas ventosae* str KM024, incubated in MJYTGL medium pH 10.5, (C) *Halomonas hamiltonii* str KM007, incubated in MB2216, (D) *Halomonas hamiltonii* str KM072, incubated in MB2216, (E) *Halomonas stevensii* str KM051, incubated in MB2216, (F) *Halomonas johnsoniae* str KM073, incubated in MB2216, (G) *Halomonas shengliensis* str KM026, incubated in MJYTGL medium pH 10.5 (H) *Demequina salsinemoris* str KM012, incubated in MB2216. Average Optical Density at 600nm (OD600) of 3 replicates vs Time. Errors bars represent standard deviations calculated from the results of 3 replicates. All incubations were performed at 23°C.

A

Marinobacter alkaliphilus str KM021

B

Marinobacter alkaliphilus str ODP1200D1.5

C

Marinobacter hydrocarbonoclasticus str KM034

Supplementary Figure 2. Pressure Growth Curves for *Marinobacter* isolates. (A) *Marinobacter hydrocarbonoclasticus* str KM034, incubated in MB2216, (B) *Marinobacter alkaliphius* str KM021, incubated in MJYTGL medium pH 9, (C) *Marinobacter alkaliphilus* str ODP1200D-1.5, incubated in MJYTGL medium pH 9. Average Optical Density at 600nm (OD600) of 3 replicates vs Time. Errors bars represent standard deviations calculated from the results of 3 replicates. All incubations were performed at 23°C.
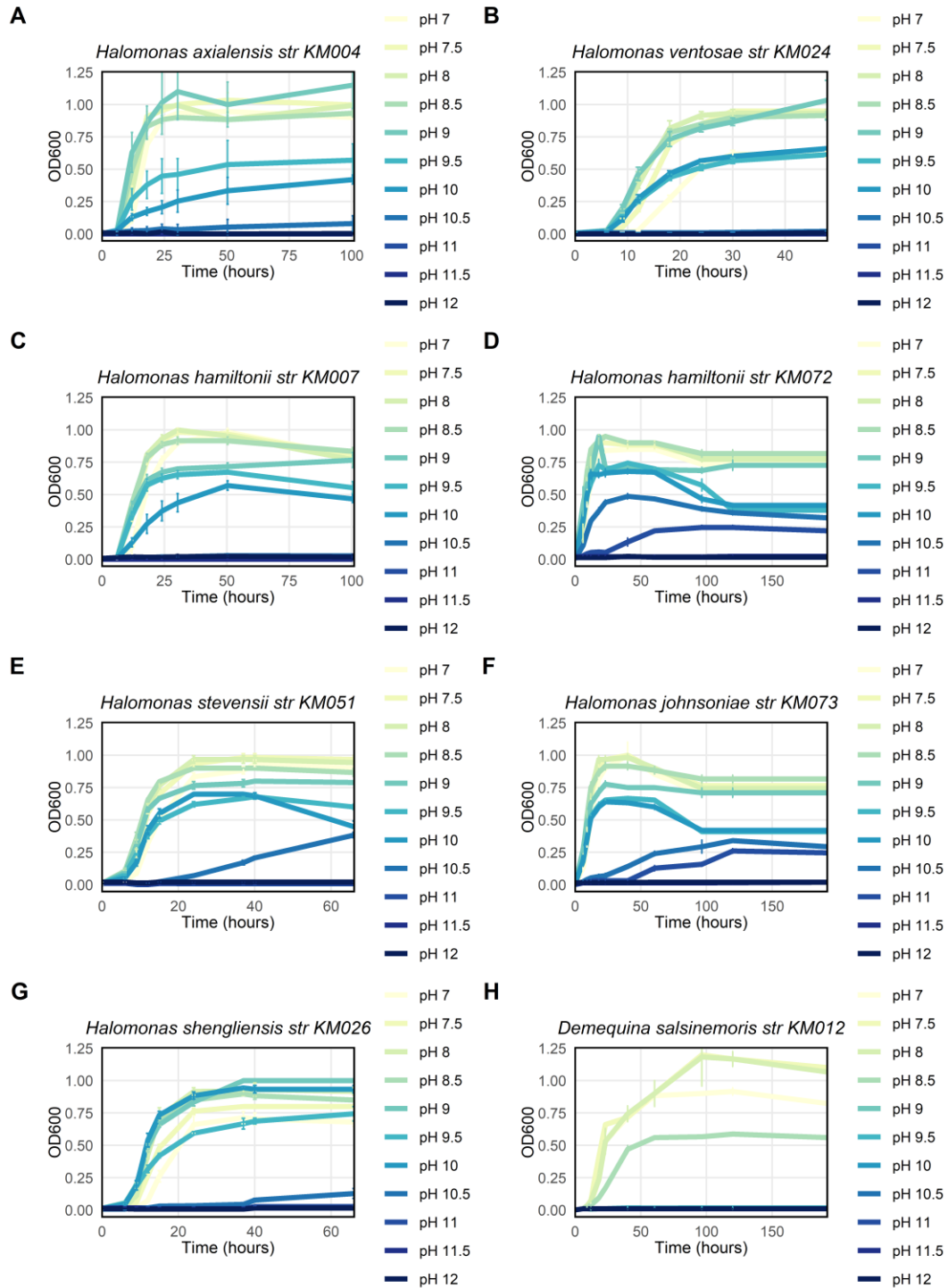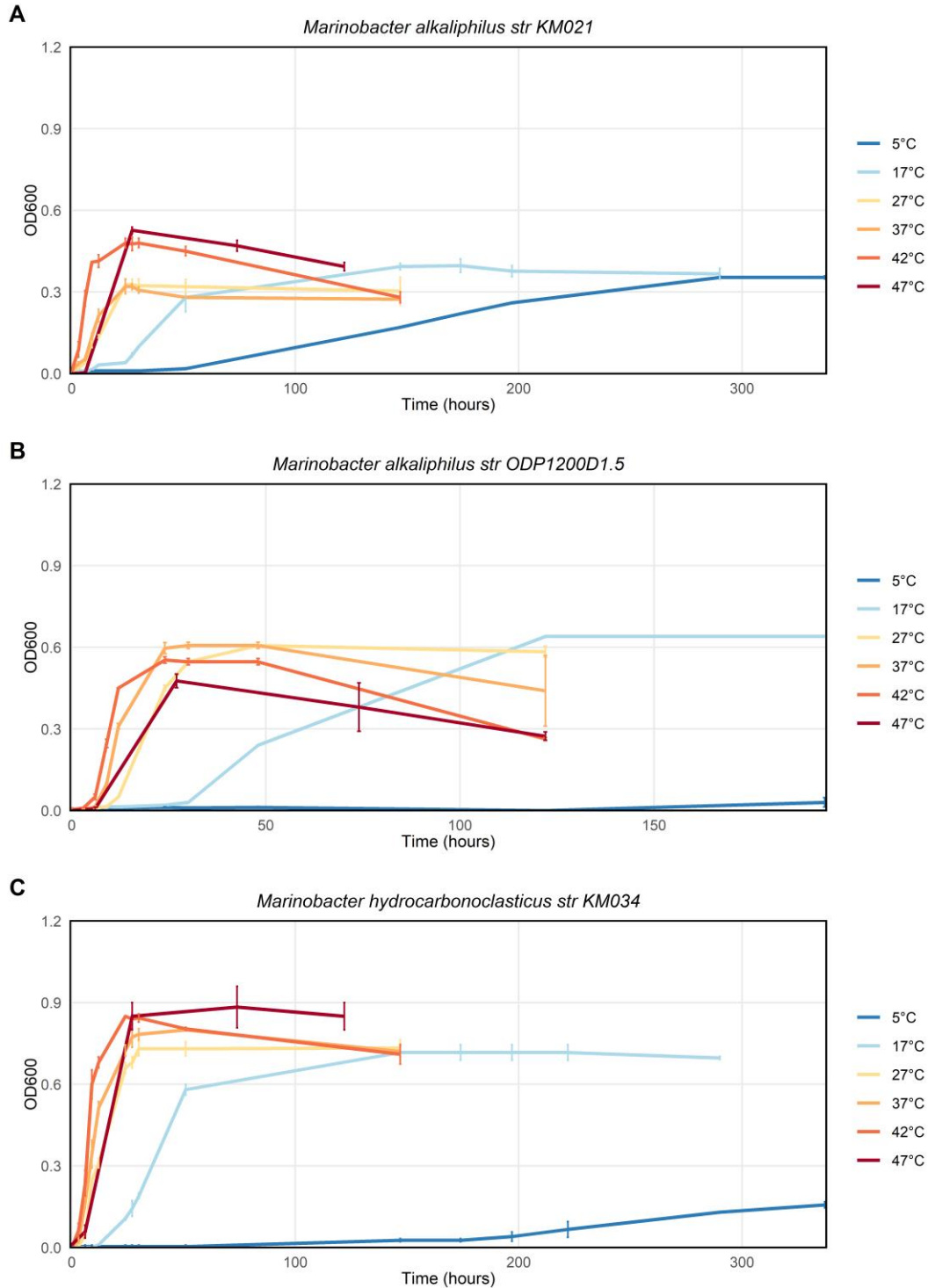
Supplementary Figure 3. pH Growth Curves for *Halomonas* and *Demequina* isolates. (A) *Halomonas axialensis* str KM004, (B) *Halomonas ventosae* str KM024, (C) *Halomonas hamiltonii* str KM007, (D) *Halomonas hamiltonii* str KM072, (E) *Halomonas stevensii* str KM051, (F) *Halomonas johnsoniae* str KM073, (G) *Halomonas shengliensis* str KM026, (H) *Demequina salsinemoris* str KM012. Average Optical Density at 600nm (OD600) of 3 replicates vs Time. Errors bars represent standard deviations calculated from the results of 3 replicates. All incubations were performed in MJYTGL medium in shaking incubators at 30°C.

63

A

Marinobacter alkaliphilus str KM021

B

Marinobacter alkaliphilus str ODP1200D1.5

C

Marinobacter hydrocarbonoclasticus str KM034
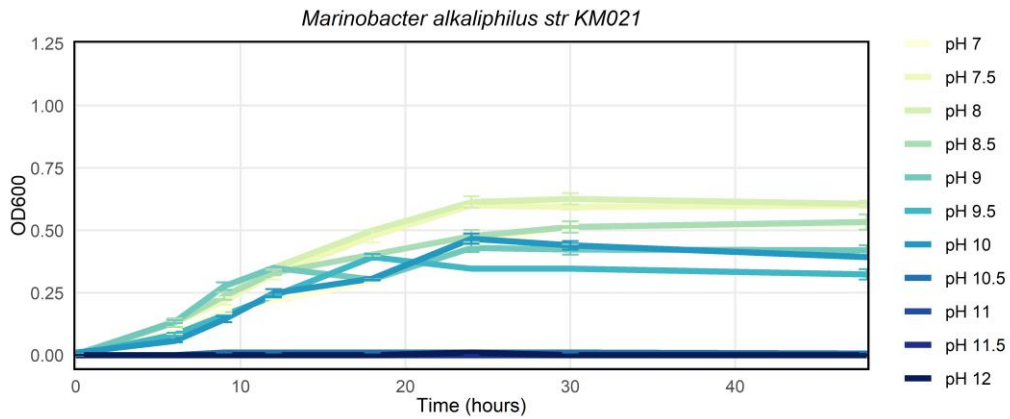
Supplementary Figure 4. pH Growth Rates for *Marinobacter* isolates. (A) *Marinobacter hydrocarbonoclasticus* str KM034, (B) *Marinobacter alkaliphilus* str KM021, (C) *Marinobacter alkaliphilus* str ODP1200D-1.5. Average Optical Density at 600nm (OD600) of 3 replicates vs Time. Errors bars represent standard deviations calculated from the results of 3 replicates. All incubations were performed in MJYTGL medium in shaking incubators at 30°C.
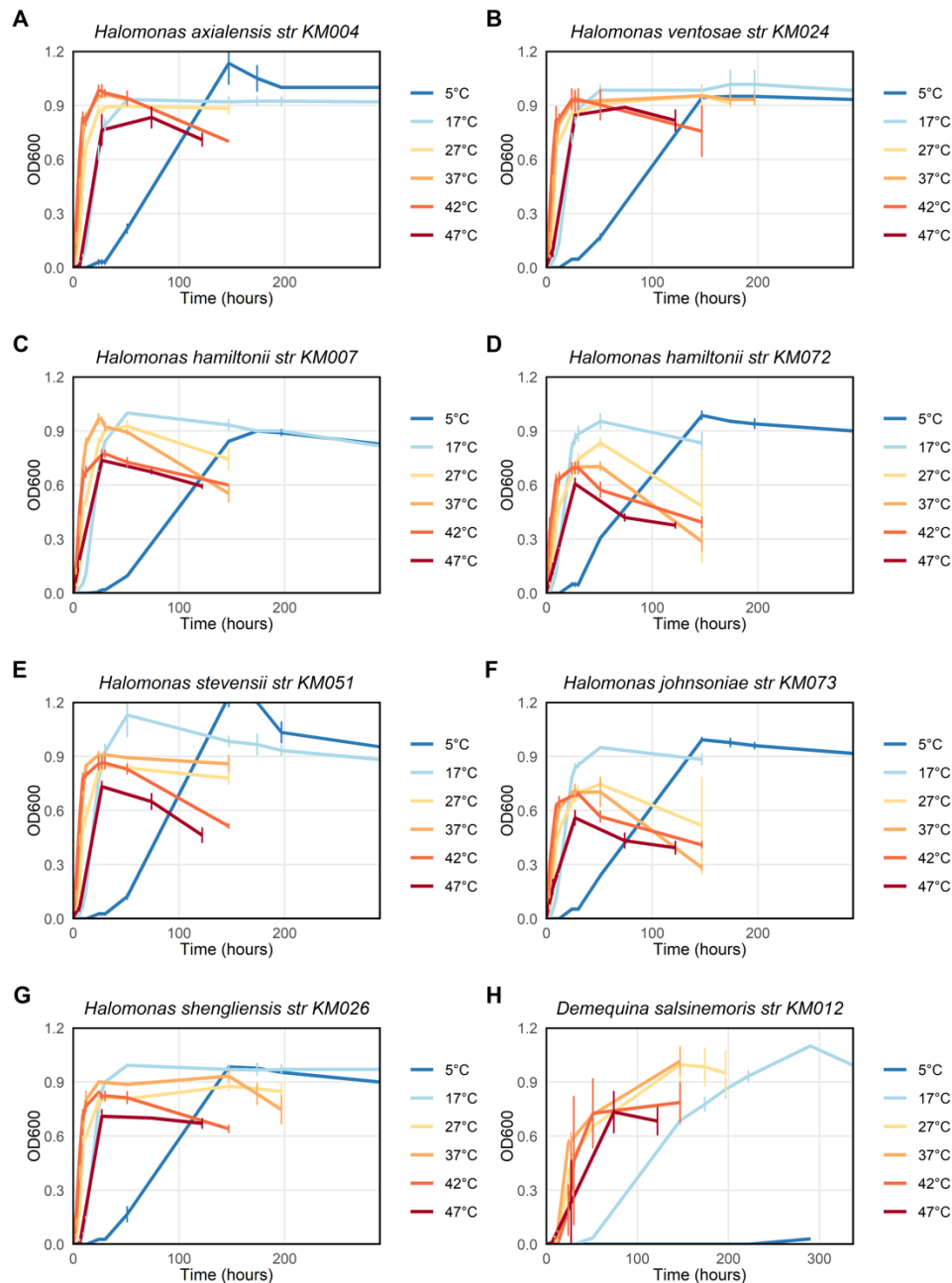
Supplementary Figure 5. Temperature Growth Curves for *Halomonas* and *Demequina* isolates. (A) *Halomonas axialensis* str KM004, incubated MJYTGL medium 8.5, (B) *Halomonas ventosae* str KM024, incubated in MJYTGL medium pH 8.5, (C) *Halomonas hamiltonii* str KM007 incubated in MJYTGL medium pH 7.5, (D) *Halomonas hamiltonii* str KM072, incubated in MJYTGL medium pH 9, (E) *Halomonas stevensii* str KM051, incubated in MJYTGL medium pH 8.5, (F) *Halomonas johnsoniae* str KM073, incubated in MJYTGL medium pH 9, (G) *Halomonas shengliensis* str KM026, incubated in MJYTGL medium pH 8, (H) *Demequina salsinemoris* str KM012, incubated in MJYTGL medium pH 7.5. Average Optical Density at 600nm (OD600) of 3 replicates vs Time. Errors bars represent standard deviations calculated from the results of 3 replicates. All incubations were performed in shaking incubators.

Supplementary Figure 6. Temperature Growth Curves for *Marinobacter* isolates. (A) *Marinobacter hydrocarbonoclasticus* str KM034, incubated in MJYTGL medium pH 7, (B) *Marinobacter alkaliphilus* str KM021, incubated in MJYTGL medium pH 9, (C) *Marinobacter alkaliphilus* str ODP1200D-1.5, incubated in MJYTGL medium pH 9. Average Optical Density at 600nm (OD600) of 3 replicates vs Time. Errors bars represent standard deviations calculated from the results of 3 replicates. All incubations were performed in shaking incubators.
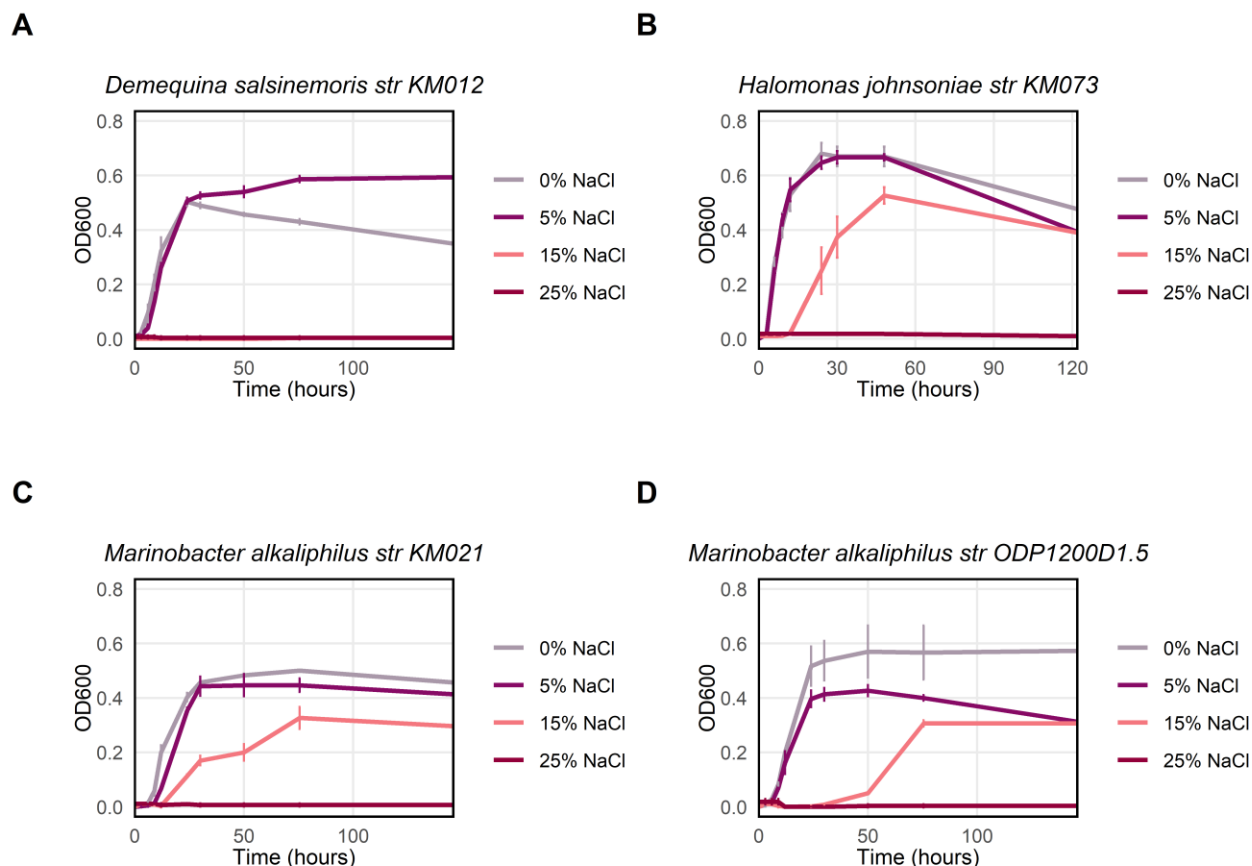
Supplementary Figure 7. NaCl Tolerance Growth Curves. (A) *Halomonas johnsoniae* str KM073, incubated in MJYTGL medium pH 9, (B) *Demequina salsinemoris* str KM012, incubated in MJYTGL medium pH 7.5, (C), *Marinobacter alkaliphilus* str KM021, incubated in MJYTGL medium pH 9, (D) *Marinobacter alkaliphilus* str ODP1200D-1.5, incubated in MJYTGL medium pH 9. The MJYTGL media contained a varying concentration of NaCl from 0% - 25% instead of 3% NaCl. Average Optical Density at 600nm (OD600) of 3 replicates vs Time. Errors bars represent standard deviations calculated from the results of 3 replicates. All incubations were performed at 37°C in shaking incubators.
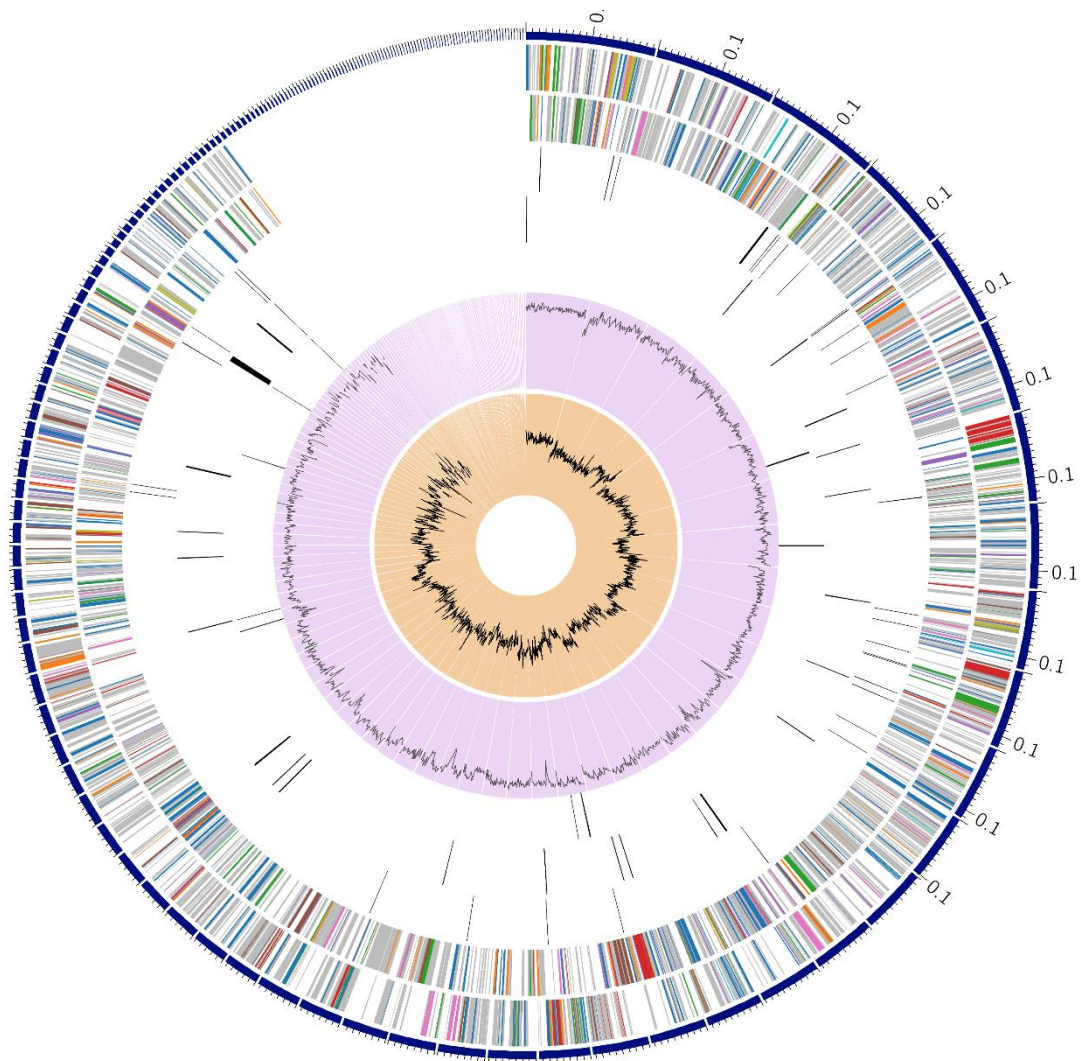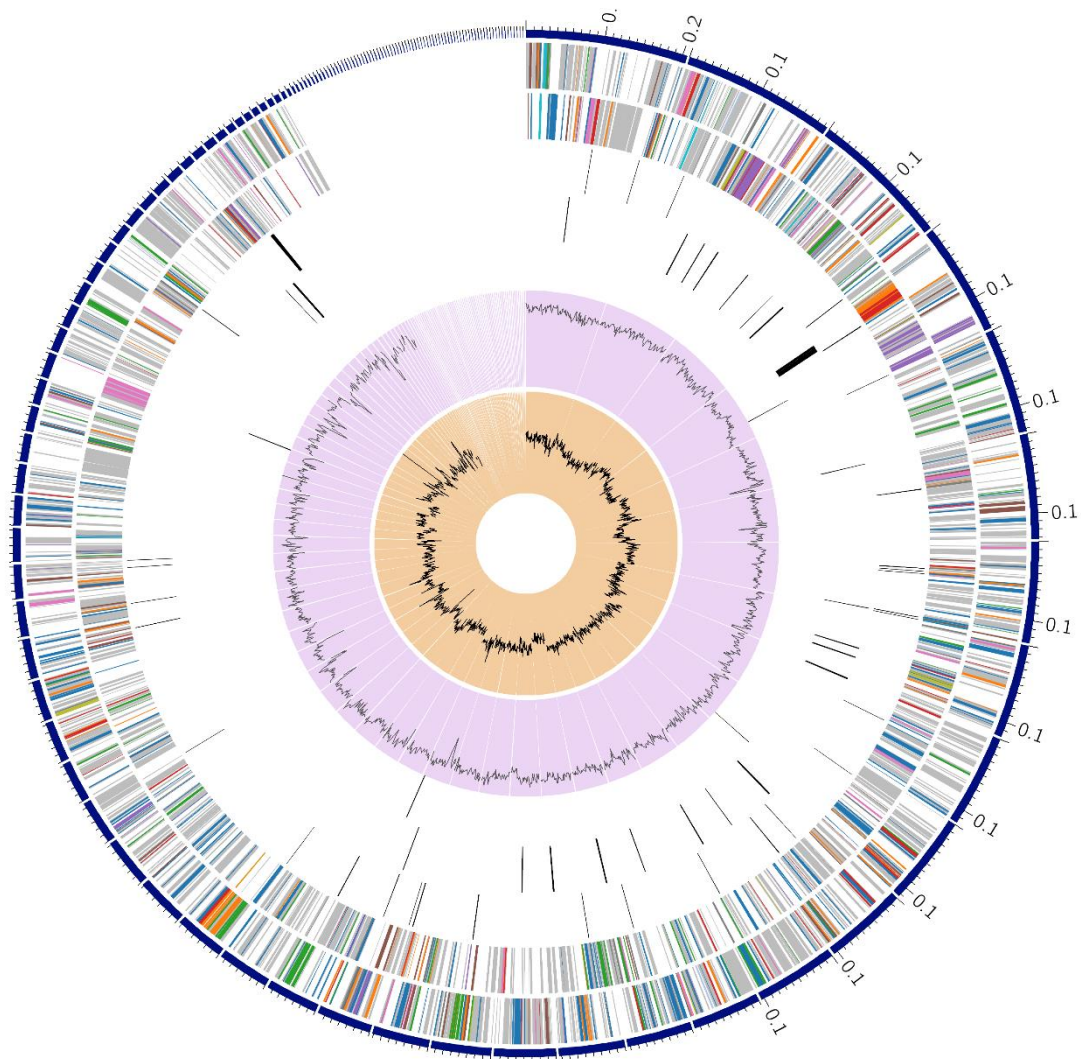
Supplementary Figure 8. Draft Genome of *Halomonas johnsoniae* str KM073. Rings from outermost to innermost represent: contigs, coding sequences on the forward strand, coding sequences on the reverse strand, RNA genes, coding sequences with homology to known virulence factors, GC content, and GC skew. Colors of the coding sequences on the forward and reverse strands correspond to subsystems in the PATRIC database.
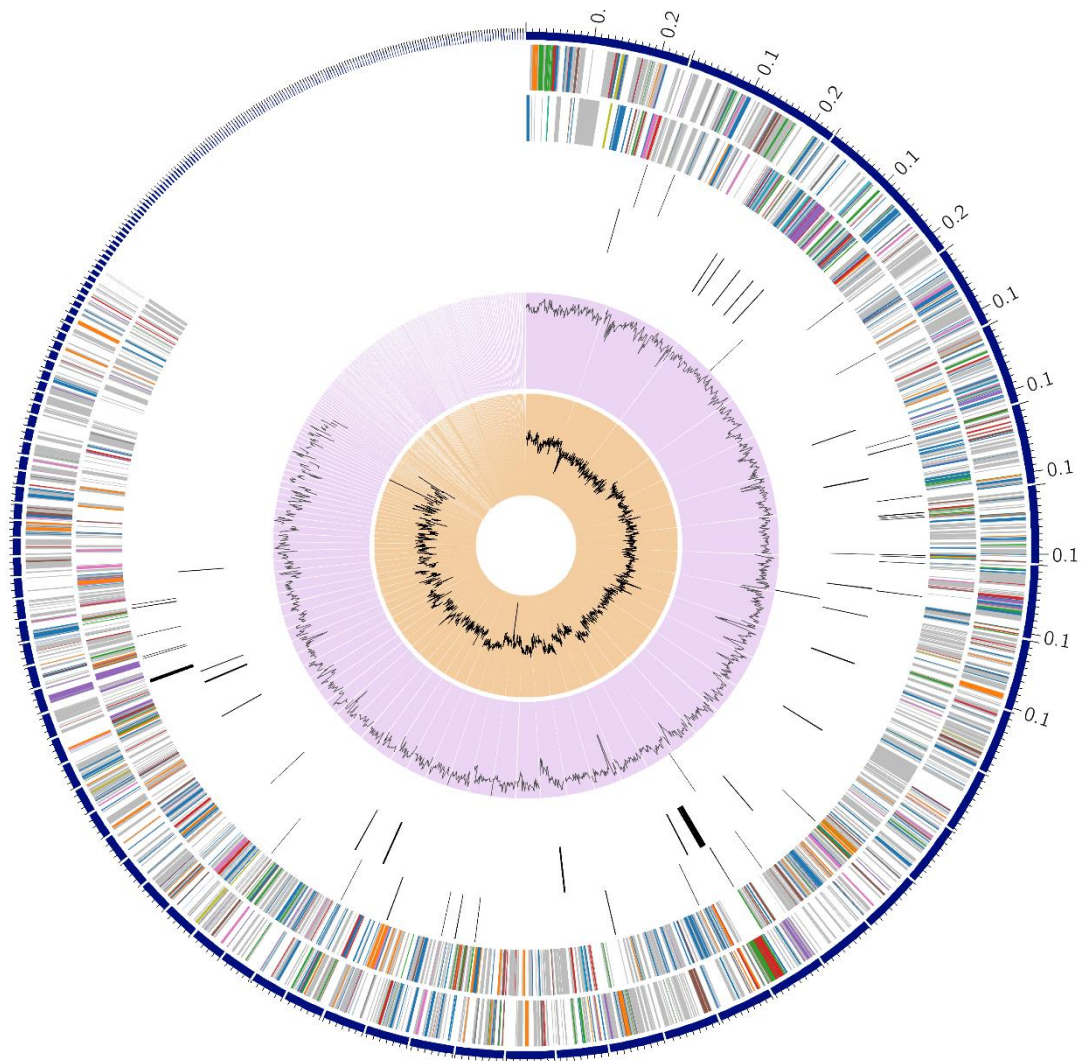
Supplementary Figure 9. Draft Genome of *Halomonas stevensii* str KM051. Rings from outermost to innermost represent: contigs, coding sequences on the forward strand, coding sequences on the reverse strand, RNA genes, coding sequences with homology to known virulence factors, GC content, and GC skew. Colors of the coding sequences on the forward and reverse strands correspond to subsystems in the PATRIC database.
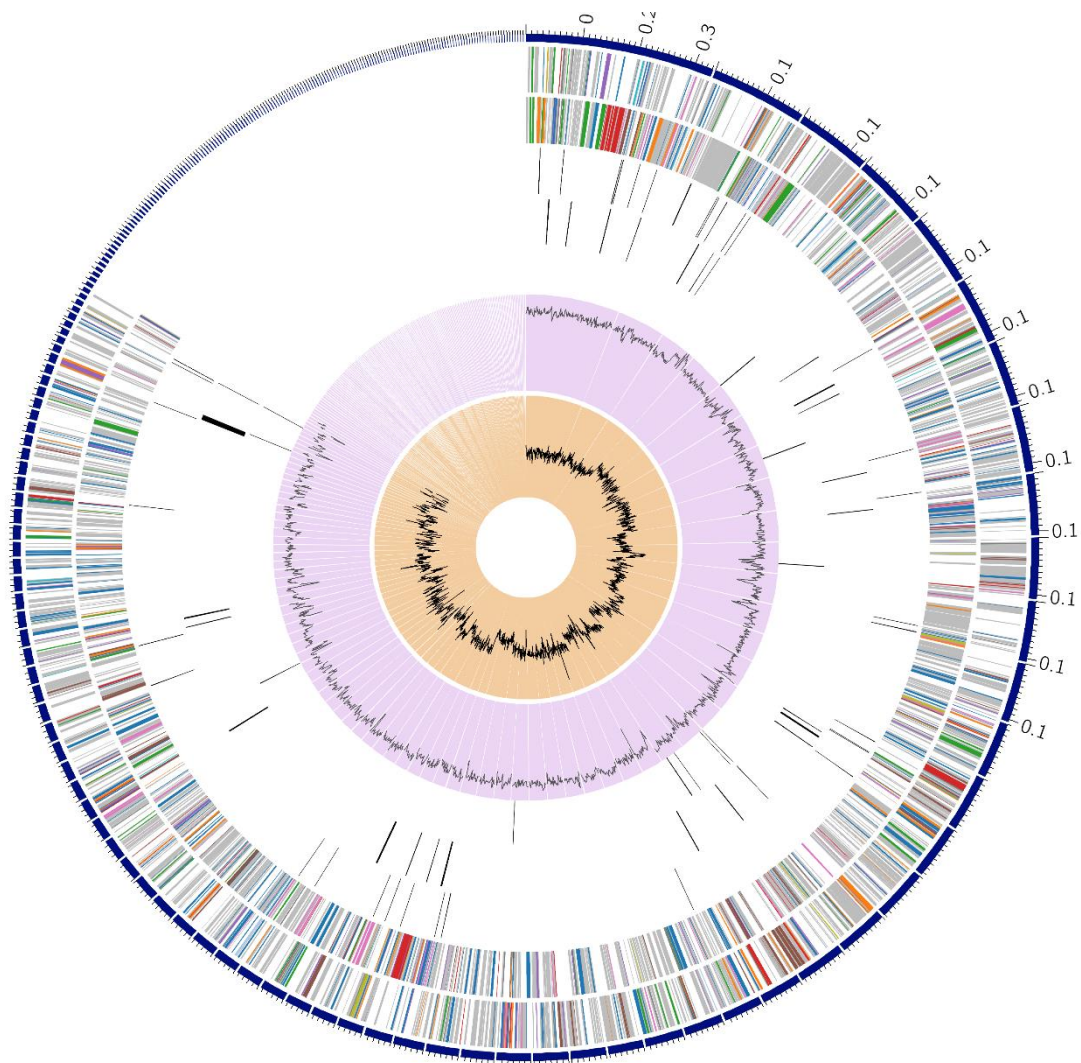
Supplementary Figure 10. Draft Genome of *Marinobacter alkaliphilus* str KM021. Rings from outermost to innermost represent: contigs, coding sequences on the forward strand, coding sequences on the reverse strand, RNA genes, coding sequences with homology to known virulence factors, GC content, and GC skew. Colors of the coding sequences on the forward and reverse strands correspond to subsystems in the PATRIC database.

Supplementary Figure 11. Draft Genome of *Marinobacter alkaliphilus* str ODP1200D-1.5. Rings from outermost to innermost represent: contigs, coding sequences on the forward strand, coding sequences on the reverse strand, RNA genes, coding sequences with homology to known virulence factors, GC content, and GC skew. Colors of the coding sequences on the forward and reverse strands correspond to subsystems in the PATRIC database.

Supplementary Table 1. Summary of Characteristics of sediment samples that yielded isolates used in this study. Estimated temperatures and pressures were calculated based on temperature and pressure at slab and at the seafloor, the data for South Chamorro Seamount were obtained from Salisbury et al. (2002), all other data were obtained from Fryer et al. (2018b).

Characteristic	Asùt Tesoru Sample 1	Asùt Tesoru Sample 2	Asùt Tesoru Sample 3	Fantangisña	South Chamorro
sample depth (mbsf)	10.4 - 11.3	0 - 0.91	29.59 - 30.97	14.6 - 16	1.45
total sample depth (m)	3369.32	2199.8	2229.39	2032.82	~2951.45
measured in-situ pH	8.35	9.01	8.7	7.87	9.5
distance to trench (km)	72	72	72	62	78
depth to slab (km)	18	18	18	14	18
slab temperature (°C)	~250	~250	~250	~150	250 - 350
estimated in-situ temperature (°C)	2.35	2.21	2.62	3.66	1.7
estimated in-situ pressure (MPa)	34.3	22.42	22.95	20.85	-

Supplementary Table 2. List of Buffers used for each pH. Final concentration was 10mM in all cases.

Medium	Buffer
Standard MJYTGL pH 7	HEPES
Standard MJYTGL pH 7.5	HEPES
Standard MJYTGL pH 8	Tris-HCl
Standard MJYTGL pH 8.5	Tris-HCl
Alkaline MJYTGL pH 9	Glycine-NaOH
Alkaline MJYTGL pH 9.5	Glycine-NaOH
Alkaline MJYTGL pH 10	CABS
Alkaline MJYTGL pH 10.5	CABS
Alkaline MJYTGL pH 11	CABS
Alkaline MJYTGL pH 11.5	CABS
Alkaline MJYTGL pH 12	CABS

Supplementary Table 3. Isolation Conditions for each of the 10 isolates selected for characterization.

Isolate	Medium (1.7% agar)	Temperature (°C)	Aerobic/Anaerobic	Sediment Sample Origin
H. axialensis str KM004	MB 2216	5	aerobic	Asùt Tesoru
H. ventosae str KM024	MJYTGL pH 10.5	23	anaerobic	Asùt Tesoru
H. hamiltonii str KM007	MB 2216	23	aerobic	Asùt Tesoru
H. hamiltonii str KM072	MB 2216	23	aerobic	Fantangisña
H. stevensii str KM051	MB 2216	23	aerobic	Asùt Tesoru
H. johnsoniae str KM073	MB 2216	37	aerobic	Fantangisña
H. shengliensis str KM026	MJYTGL pH 10.5	23	anaerobic	Asùt Tesoru
D. salsinemoris str KM012	MB 2216	23	anaerobic	Asùt Tesoru
M. alkaliphilus str KM021	MJYTGL pH 10.5	37	aerobic	Asùt Tesoru
M. hydrocarbonoclasticus str KM034	MB 2216	37	aerobic	Asùt Tesoru