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[Mini review] metagenomic studies of the Red Sea

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**Title:**

[Mini Review] Metagenomic studies of the Red Sea

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**Abstract**

Metagenomics has significantly advanced the field of marine microbial ecology, revealing the vast diversity of previously unknown microbial life forms in different marine niches. The tremendous amount of data generated has enabled identification of a large number of microbial genes (metagenomes), their community interactions, adaptation mechanisms, and their potential applications in pharmaceutical and biotechnology-based industries. Comparative metagenomics reveals that microbial diversity is a function of the local environment, meaning that unique or unusual environments typically harbor novel microbial species with unique genes and metabolic pathways. The Red Sea has an abundance of unique characteristics; however, its microbiota is one of the least studied amongst marine environments. The Red Sea harbors approximately 25 hot anoxic brine pools, plus a vibrant coral reef ecosystem. Physiochemical studies describe the Red Sea as an oligotrophic environment that contains one of the warmest and saltiest waters in the world with year-round high UV radiations. These characteristics are believed to have shaped the evolution of microbial communities in the Red Sea. Over-representation of genes involved in DNA repair, high-intensity light responses, and osmolyte Cl oxidation were found in the Red Sea metagenomic databases suggesting acquisition of specific environmental adaptation by the Red Sea microbiota. The Red Sea brine pools harbor a diverse range of halophilic and thermophilic bacterial and archaeal communities, which are potential sources of enzymes for pharmaceutical and biotechnology-based application. Understanding the mechanisms of these adaptations and their function within the larger ecosystem could also prove useful in light of predicted global warming scenarios where global ocean temperatures

are expected to rise by 1-3 °C in the next few decades. In this review, we provide an overview of the published metagenomic studies that were conducted in the Red Sea, and the bio-prospecting potential of the Red Sea microbiota. Furthermore, we discuss the limitations of the previous studies and the need for generating a large and representative metagenomic database of the Red Sea to help establish a dynamic model of the Red Sea microbiota.

**Keywords:** Red Sea, metagenomics, microbiota, unique niches, novel species and genes, biotechnology, dynamic models.

### **Introduction**

In recent years, significant advances were made in the field of environmental microbiology due to the advent of next-generation sequencing technology (NGS), which facilitated the establishment of metagenomics. Combined with NGS, metagenomics provides a powerful platform to examine and compare microbial fingerprints of different environments, their adaptation mechanisms, host microbe interactions, and microbial evolution. Metagenomic studies of water and soil provided significant insights into previously unknown terrestrial and marine microorganisms (Daniel, 2005; Simon and Daniel, 2011). Novel microbes, their genomes, and adaptation mechanisms were identified in extreme and inhospitable environments such as acid mine drainage sites (Tyson et al., 2004), deep-sea hyper-saline brine pools (Wang et al., 2013), arctic soil (Lipson et al., 2013), and glaciers (Simon et al., 2009), providing evidence for extreme genomic plasticity and adaptation mechanisms of these microorganisms.

A substantial number of metagenomic-based studies have been conducted on marine environments, for several reasons. Water covers approximately three quarters of our planet, and is home to a vast range of microbial life forms that play essential roles in global ecosystems. The majority of marine niches are still unexplored, but those that were examined revealed a diversity of novel microbes with unique functions. Previous studies also strongly suggest that microbial diversity and functions are directly linked with their local environments, justifying examination of new and unusual niches for novel genes and species. In addition, marine microbes appear to have great prospect in biotechnology applications and many studies are underway to tap into these natural resources. Finally, monitoring changes in marine microbial diversity would provide an accurate index of environmental health and ecosystem sustainability.

Despite its unique physical and chemical attributes, the Red Sea is one of the least explored oligotrophic (low nutrients) marine environments. The Red Sea is characterized by its high year round temperature, high UV radiation, high salinity, low nutrients, extreme niches such as hot brine pools, and a vibrant coral reef ecosystem. The few metagenomic studies that were conducted on the Red Sea revealed that its microbiota is distinct from those found in other marine environments (Thompson et al., 2013). For example, the Red Sea hot brine pools harbor a diversity of halophiles, thriving in conditions thought to be inhospitable to organic life (Bougouffa et al., 2013). It is expected that such extremophiles have developed metabolic pathways and enzymes with potential biotechnology-based applications (Mohamed et al., 2013; Sayed et al., 2014). These pools, like many other unique niches in the Red Sea, remain understudied.

In this review, we summarize the published metagenomic works conducted in the Red Sea, their significant findings, and the bio-prospecting potential of the Red Sea microbiota. We discuss the limitations of previous studies, and the need for spatio-temporal models of the Red Sea microbiota to gain insight into their community stability and dynamics. We also preview the scope of the Red Sea metagenomics explorations that are being conducted by the authors of this paper and colleagues at King Abdullah University of Science and Technology (KAUST).

### **The Red Sea**

In geological terms, the Red Sea rift began to develop approximately 30 million years ago (Dixon, 1990) through divergent movement and splitting apart of African and Arabian lithosphere plates; this was followed by thinning out of the continental plates accompanied by large number of volcanic activities, development of an active rift, and formation of typical oceanic crust in its axial zone (Gurvich, 2006). The maximum depth of the rift is approximately 2900 m in the South and 1500 m in North. The boundary between the thin continental plates and the oceanic crust extends along the central axis of the Red Sea basin, the area containing large number of hot brine pools.

The Red Sea is the world northern most tropical sea located between arid and semi-arid deserts. It has a surface area of roughly  $\sim 4.6 \times 10^5 \text{ km}^2$ , volume of  $\sim 2.5 \times 10^5 \text{ km}^3$ , length of  $\sim 2000 \text{ km}$ , maximum width of 355 km, and a maximum depth of 3039 m (Rasul et al., 2015). Three distinct depth zones can be found within the Red Sea: 1)

Shallow zone along its shoreline, with depth of less than 100 m covering approximately 40% of the sea; 2) Deep zone ranging from 500 m to 1000 m; and 3) Deeper zone along its central axis with depth ranging from 1000 m to ~2900 m where the majority of brine pools are located (Rasul et al., 2015). The Red Sea is relatively isolated from the rest of world oceans (Thompson, 2014), connected to them only by a shallow strait (Bab-el-Mandeb, 310 m deep) in the South and an even shallower canal (Suez Canal, 25 m deep) in the North. The Strait of Bab-el-Mandeb connects the Red Sea to the Gulf of Aden in the South and the Suez Canal connects it to the Mediterranean Sea in the North (Fig.1). The Red sea is characterized as an oligotrophic environment with year-round UV irradiation and high surface temperature that can reach up to 34 °C during summer with only minor variations during winter. It contains one of the world saltiest water with the surface salinity ranging from 36 psu (south) to 41 psu (north), with an average salinity of 40 psu (Ngugi et al., 2012). The high salinity is attributed to the high rate of evaporation, the low annual precipitation, lack of river inflow, and relative isolation from the world body of ocean.

The Red Sea contains approximately 25 deep-sea brine pools, which contain mineralized hot brine with metalliferous deposits generated from dissolution of Miocene evaporites and hydrothermal activities deep in the sea floor. These brine pools are considered extreme environments with up to seven times higher salinity than the surrounding deep sea water, high temperatures (up to 60 °C and more), low pH, high concentrations of dissolved metals such as Fe, Mn, Zn, Cu, Pb, Co, Ba, Si, Li, and low concentration or complete absence of dissolved oxygen (Gurvich, 2006). The composition of sediments, percent salinity, pH, and temperature varies between different

brine pools; even within the same pool, vertical stratification could occur resulting in distinctive layers of similar temperatures and salinity, beginning at the sea water-brine interface (Antunes et al., 2011). Due to their extreme nature, the Red Sea brine pools and their sediments can provide a perfect niche to examine extremophiles for their adaptation mechanisms and bio-prospecting potential.

Another unique feature of the Red Sea is its coral reef ecosystem. The Red Sea fringing coral reef complex extend some 2000 kilometers along the shoreline, and provide the main source of food and shelter for a large number of species of fish and invertebrate. The Red Sea also contains numerous offshore reefs with wide range of reef morphology that include barrier reefs, atoll reefs, patch reefs, and platform reefs. These offshore reef formations are very unusual and do not follow the general reef classification schemes. The coastal and offshore reefs contain some of the most diverse corals with an unusual ability to survive in one of the world saltiest and hottest waters. Increased overpopulation and industrialization along coastal regions, and the resulting anthropogenic activities have posed a significant threat to the Red Sea coral reef habitats specially those growing in the coastal regions.

### **The Red Sea Metagenomics**

Metagenomics is a powerful molecular and bioinformatics-based approach that enables characterization of the entire microbiome of a given environment by sequencing microbial DNA recovered directly from the environment without prior cultivation. Metagenomics can help establish microbial fingerprints of various environments and identify the microbial genes that carryout specific functions within the community

metagenome. In addition, since microbial diversity is a function of the local environment, metagenomics-based approaches can identify the environmental factors responsible for shaping different microbial community structures. Accordingly, phenotypic changes in response to drastic environmental changes can be identified. Finally, metagenomics-based approaches can help identify novel microbial genes for biotechnology-based applications.

Surprisingly, few metagenomic explorations have been conducted in the Red Sea despite its unique characteristics: an oligotrophic environment containing one of the world saltiest and hottest waters potentially harboring microbes with unique adaptation mechanisms. Temperature gradient, salinity, nutrient availability, and dissolved oxygen (DO) concentration varies considerably along the depth of the Red Sea, which most likely impacts the vertical distribution of microorganisms, their abundances, and community composition. A recent metagenomic analysis of the Red Sea water columns overlaying two brine pools, namely Discovery Deep and Atlantis II, showed that although similar microbial communities were present in the surface water (20m and 50m depth), very distinct communities existed in the deeper columns (200m and 1500m depth) suggesting vertical stratification of microbial communities along the sea depth (Qian et al., 2011). Interestingly the same depth but different locations showed similar microbial profile. As expected, higher density and diversity of microbes were found in the upper layers, possibly due to increased light, temperature, DO, and primary productivity. Cyanobacteria were shown to be the most dominant bacteria in the surface water whereas Proteobacteria, in particular the gamma subdivision, became dominant in the deeper layers (200m and 1500m). While halophilic Archaea Haplobacteriales dominated the

upper layers, the deeper layers were enriched with anaerobic sulfur-dependent heterotrophic Archaea Desulfurococcales. Abundance of Desulfurococcales in the deeper layers could be due to the high sulfur concentration (Gurvich, 2006) and low DO in these layers, where the Archaea is likely involved in sulfur reduction and cycling. The data also revealed that the bacterial community composition in Red Sea were different than those found in other marine environments, and that environmental adaptation played a significant role in vertical distribution of microbial communities along the Red Sea columns. Extremely diverse microbial communities are found in the Red Sea relative to other hyper-saline marine environments (Qian et al., 2011). The Red Sea microbial community composition resembles those found in North Pacific Ocean with respect to adaptation to high-intensity light, the Mediterranean Sea for adaptation to high salinity, and the Mediterranean and Sargasso Seas for adaptation to low phosphorus concentration (to some degree) (Thompson, 2014). Physicochemical studies describe the Red Sea as an oligotrophic environment with low nitrogen and phosphorus contents, high salinity, high temperature, and high UV radiation. These characteristics are believed to have shaped the evolution of the microbial communities in the Red Sea. In the Red Sea metagenomic databases, Thomson et al (Thompson et al., 2013) found over-representation of genes involved in DNA repair, high-light responses, and osmolyte C1 oxidation, suggesting acquisition of specific environmental adaptation by the Red Sea microbiota.

Eder et al (Eder et al., 1999) were one of the first to characterize the microbial diversity in the Red Sea brine pools using molecular approaches. The authors examined microbial community structure of the sediments in Kebrit Deep brine pool by 16S rRNA gene PCR amplification and found novel 16S sequences belonging to bacteria and

Archaea that showed no close associations with available sequences in the databases. Their findings suggested that the Red Sea brine pools could harbor novel microbial species. The interface between brine pools and the seawater (BSI) is a transition region containing distinct microbial communities. Guan et al (Guan et al., 2015) evaluated the relative abundance and phylogenetic composition of bacteria and Archaea in BSI of five different Red Sea brine pools, namely Atlantis II, Discovery Deep, Erba Deep, Kebrit Deep, and Nereus Deep, and found increased abundance and diversity of bacteria over Archaea. Phylogenetic analysis of genes involved in methanogenesis and sulfate reduction demonstrated higher diversity of sulfate-reducing communities relative to the methanogenic communities in all five locations. Brine-sea water interface of Kebrit Deep in Northern Red Sea was shown to harbor novel halophiles associated with the genus *Halanaerobium* (Eder et al., 2001), potentially contributing to the degradation of enriched organic matters at the BSI. Wang et al (Wang et al., 2013) compared microbial community profile of two adjacent Red Sea brine pools, Discovery Deep and Atlantis II, and showed that the metagenome of the two brine pools were very distinct; while the Atlantis II brine pool metagenome was heavily represented by metal reducing heterotrophs that relied, almost entirely, on organic compounds in the surrounding water, the Discovery brine pool contained autotrophs capable of CO<sub>2</sub> fixation and methane oxidation, and functioned as primary producers. Siam et al (Siam et al., 2012) compared the microbial community composition of the sediments in Atlantis II, Discovery Deep, and Chain Deep brine pools. Although the metagenome of all three pools had very similar distribution of rare and abundant microbes, the deepest sulfur-rich sediments from Atlantis II and the nitrogen-rich sediments from Discovery each showed distinct Archaeal

and bacterial profiles: the Operational Taxonomic Units (OTUs) found in Atlantis II clustered mainly with Archaeal and bacterial communities involved in sulfur reduction, while those from Discovery Deep clustered with nitrogen cycling. The ammonia oxidizing Archaeal (AOA) and bacterial communities play significant roles in marine nitrogen cycling. Ngugi et al (Ngugi et al., 2015) examined the presence of ammonia-oxidizing microbes in several Red Sea BSI (with up to 18.2% salinity) and revealed that the Archaeal communities in these BSI consisted mainly of a single but highly abundant AOA phylotype *Nitrosopumilus*-like phylotype (RSA3). Interestingly no ammonia-oxidizing bacteria were found. Analysis of the single amplified genome of the AOA from one of these BSI revealed high degree of genetic diversity and metabolic plasticity associated with adaptation in such extreme environment. The presence of a putative proline-glutamate ‘switch’ in the genome of these Archaea, for example, suggests adaptation to high salinity. Further explorations of these and other brine pools in the Red Sea can provide more detailed insights into their microbial community profile, and the metabolic pathways used for adaptation in extreme conditions.

Diverse communities of microbes dwell within the Red Sea coral reef ecosystem. Corals live in symbiotic relationship with endosymbiotic dinoflagellate *symbiodinium* (Baker, 2003; Stat et al., 2006), Archaea, bacteria, fungi, and viruses (Rohwer et al., 2002; Rosenberg et al., 2007; Ainsworth et al., 2010). Although the essential role of *symbiodinium* in coral fitness and their major role in carbon fixation is well established, the significance of coral microbiota in maintaining coral health and survival is not well understood. Studies suggest that coral symbionts play important roles in carbon and nitrogen fixation, coral defense (antibiotic production), and other aspects of coral health

and survival (Williams et al., 1987; Rohwer et al., 2002; Lesser et al., 2004; Wegley et al., 2007). Despite these indications, only few studies have examined the microbiota of Red Sea corals. A recent study by Bayer et al (Bayer et al., 2014) revealed that *Endozoicomonas* bacteria was intimately associated with the Red Sea coral *Stylophora Pistillata*, and other corals worldwide. Comparison of the microbiome of healthy (asymptomatic) and diseased (symptomatic) Caribbean corals showed that the symptomatic coral tissue had lost their *Endozoicomonas* (Meyer et al., 2014). Interestingly, the diseased coral tissue also harbored potential opportunistic pathogens such as *Alternaria*, *Stenotrophomonas*, and *Achromobacter*. It is possible that the loss of *Endozoicomonas* contributed to overtake of the lesion by opportunistic pathogens. Sponges are one of the key players in maintaining a healthy coral reef ecosystem in part through recycling of the nutrients released by corals. Similar to corals, sponges have developed symbiotic relationships with microbes to help them adapt to harsh environmental conditions. The unusual high tolerance of *Theonella swinhoei* to arsenate and arsenite, for example, is attributed to its microbiome (Keren et al., 2015). Gao et al (Gao et al., 2014a; Gao et al., 2015) examined the microbial diversity of healthy vs. diseased sponges collected from the Red Sea and reported a drastic shift in microbiome of abnormal sponge tissue. While the healthy sponge tissue showed a lower microbial diversity, the diseased tissue demonstrated a dramatic increase in microbial diversity with a decrease in sponge-specific microbial communities. In addition to their beneficial impact on the host, the host-microbe symbiotic associations are believed to have shaped the evolution of microbial symbionts. Gao et al (Gao et al., 2014b) published the first draft genome of *Candidatus Synechococcus spongiarum*, a broadly dispersed

cyanobacterial symbiont of the sponge *Carteriospongia foliascens* found in the Red Sea. Compared with free-living cyanobacterial strains, the symbiotic strain appeared to have lost partial genes encoding proteins involved in photosynthesis, DNA repair mechanism, adaptation to environmental stress, and production of capsular and extracellular polysaccharides. The rise in temperature and acidity of the ocean due to natural and anthropogenic activities continue to jeopardize the coral health globally, promoting coral bleaching and disintegration. Such damages can negatively impact the rich communities of marine life that are sheltered and nourished by these reefs. As the global temperature continues to rise, it is imperative to examine the causes of coral bleaching, and the potential role of coral microbiota in preventing or rescuing the coral reef ecosystem from such damages. Although it is very rare to observe diverse and flourishing reef ecosystems above 31 °C, the vibrant corals present in southern Red Sea have managed to acclimatize and adapt to temperatures as high as 34 °C and more. The Red Sea coral symbionts might provide an important clue to coral adaptation mechanisms to increased heat. Understanding the mechanisms of these adaptations could prove useful in light of the predicted global warming scenario where the global ocean temperature is expected to rise by 1-3 °C in the next couple of decades.

Metagenomic studies continue to unravel the mechanisms involved in microbial biodiversity and adaptation to environmental changes, in particular to extreme environments. Many of these adaptation mechanisms usually involve gain of functions that are important in metabolism and survival under extreme environments. These adaptation mechanisms have also been exploited for biotechnology-based applications.

### **Exploration of the Red Sea microbiota for biotechnology-based applications**

Metagenomics is a powerful tool used to access the genome of uncultivable microorganisms and assess their potential as sources of novel biocatalysts for biotechnology-based applications. The approach involves direct cloning of the environmental DNA into suitable hosts such as *E. coli*, functional screening (Venter et al., 2004), followed by sequencing of the positive clones to identify genes responsible for the desired phenotypes (Tyson et al., 2004; Lee and Lee, 2013). Alternatively, the shotgun metagenomic data can be mined for genes with bio-prospecting potential. Metagenomic studies led to discoveries of many novel lipolytic enzymes such as lipases (Hardeman and Sjoling, 2007; Cieslinski et al., 2009) and esterases (Henne et al., 2004; Elend et al., 2006; Chu et al., 2008); cellulases, and chitinases (Henne et al., 2004; Warnecke et al., 2007; Duan et al., 2009; Jacquiod et al., 2013); DNA polymerases (Schmidt et al., 2014); amidases used for biosynthesis of  $\beta$ -lactam antibiotics (Voget et al., 2003; Gabor et al., 2004); biocatalysts including oxidoreductases and dehydrogenases (Knietsch et al., 2003); proteases used in detergent and food industries (Henne et al., 2004; Waschkowitz et al., 2009).

Characterized as extreme environments, the Red Sea brine pools are expected to be a source of novel microbial enzymes and biomolecules that have evolved to function under extreme conditions suitable for biotechnology and industrial-based applications. Surprisingly, these pools have seldom been explored for these purposes. The few metagenomic studies conducted on these brine pools have revealed the presence of diverse and novel extremophiles. Using functional screening of metagenome of the Atlantis II brine pool in the Red Sea, Mohamed et al (Mohamed et al., 2013) identified a

novel esterase, EstATII, shown to be halotolerant (active in up to 4.5 M NaCl), thermophilic (optimal temperature of 65 °C), and resistant to heavy metals (maintained 60% activity in wide range of heavy metals). Esterases have wide range of application in agriculture, food, and pharmaceutical-based industries. Sayed et al (Sayed et al., 2014) identified a novel Mercuric Reductase (merA) in a metagenomic database from Atlantis II brine pool. The gene was subsequently cloned in *E. coli* and enzymatic characterizations revealed that the enzyme was stable and functional in the presence of high salt, high temperature, and high concentrations of Hg<sup>2+</sup>, and it effectively detoxified mercury. Interestingly, the enzyme conferred mercuric resistance to the transformed *E. coli* strain. These findings could have applications in environmental bioremediations due to heavy metal pollution. Grötzinger et al (Grotzinger et al., 2014) developed a Profile and Pattern Matching Algorithm to mine genomic databases of the Red Sea brine pools for genes encoding novel enzymes, and identified over 100 novel enzymes with high potential for industrial-based applications. Their computational-based approach improved prediction capabilities over other bioinformatics methods by decreasing the number of false positive predicted annotations of enzyme function. The study demonstrates that improved *in silico*-based screening of metagenomic databases could help cut down the number of candidate genes for gene expression profiling and characterization. Werner et al (Werner et al., 2014) sequenced the genome of Euryarchaea *Halorhabdus tiamatea* isolated from the Shaban Deep hypersaline anoxic brine pool in the Red Sea and showed that the Archaea contained large number of genes encoding glycoside hydrolases with potential industry-based applications. Enzymatic analysis of cultured extracts from the Archaea revealed higher glucosidase activity at lower oxygen concentration consistent

with the anoxic environment from which the Archaea was isolated. Philosof et al (Philosof and Beja, 2013) used metagenomics and metatranscriptomics to analyze the diversity of bacteriorhodopsins and proteorhodopsins in Gulf of Aqaba at the Northern tip of the Red Sea; their data revealed the vast diversity of bacterial, archaeal and viral rhodopsin-like genes in these waters. Microbial opsins have great potentials for use in light-controlled gene expression technologies such as optogenetics and bioelectronics (Deisseroth, 2011; Wagner et al., 2013). Marine microbes can also be important sources of bioactive compounds for treatment of cancer and other diseases. Sagar et al (Sagar et al., 2013b) studied the cytotoxic activity and anticancer potential of extracts obtained from bacteria isolated from the Red Sea brine pools Atlantis II, Discovery Deep, Kebrit Deep, Nereus Deep, and Erba Deep. The extracts from four of the isolated bacterial species (*Chromohalobacter salexigens*, *Halomonas meridiana*, *Idiomarina loihiensis*, and *Chromohalobacter israelensis*) showed promising anti-cancer potential when tested against 3 different human cancer cell lines including MCF-7, HeLa, and DU145. Similar studies using the lipophilic and hydrophilic extracts of bacteria isolated from the Red Sea brine-sea water interfaces showed promising apoptotic and cytotoxic effect against the human cancer cell lines mentioned above (Sagar et al., 2013a).

The Red Sea brine pool extremophiles appear to be a great source of enzymes and biomolecules for pharmaceutical and industrial applications. Metagenomics and synthetic biology-based approaches provide a great platform to identify candidate microbial genes amongst the community metagenome and produce them at large-scales for downstream applications.

**Discussion: Future perspectives on Red Sea microbiota research**

To date, the Red Sea metagenomic studies have provided a glimpse into the vast diversity of Red Sea microorganisms and their function. However, completing the picture of the Red Sea microbiome remains an entirely different challenge, one that studies of this kind – with limited scope and conducted in isolation – cannot address. Representing microbial profiles at a particular point in time and space cannot capture the dynamics of a marine ecosystem that is continuously changing. For example, the Red Sea experiences strong seasonal pattern in phytoplankton concentration with the maximum concentrations of Chlorophyll-a in the winter and the lowest levels in the summer (due to water column stratification) (Raitsos et al., 2013). The increase in phytoplankton concentrations in winter is attributed to the upwelling of water columns that bring nutrient-rich deep water to the surface, and the wind-induced horizontal intrusion of nutrient-rich water from the Gulf of Aden in the south. The environmental factors that drive the formation of planktonic blooms and the microbial community interactions within these blooms are not well understood. Large-scale time-series studies of Red Sea microbiota in different niches are needed to establish the rhythmic pattern (diurnal, monthly, seasonal) of microbial composition and monitor perturbations in these patterns in response to anthropogenic activities. Red Sea coastal regions have experienced a dramatic rise in pollution due to increased urbanization and industrialization in recent decades. Industrial and anthropogenic pollutions released directly into the marine environment can shape the composition of microbial communities, yet metagenomic studies of Red Sea coastal regions are substantially lacking. Mustafa et al. (Mustafa et al., 2014) conducted phylogenetic analysis of cultured microbial communities isolated from sediments in

industrial zones along the Egyptian Red Sea coast and found distinct microbial communities, primarily oil/hydrocarbon degrading bacteria and several human pathogens including known *Vibrio* and *Clostridium* species. The study suggests that changes in microbial community profile could function as an index to measure the impact of anthropogenic activities on marine environments. In addition to monitoring the coastal zone, it is important to study the Red Sea pelagic zone and identify the environmental factors that shape the composition of microbial communities in different layers in euphotic (0-200 m from surface), mesopelagic (200-1000 m deep), and bathypelagic (1000-3000 m deep) zones, and examine how the microbial community structure in different zones are impacted over time. Vertical stratification and mixing of the water column due to seasonal changes, for example, can influence the composition of microbial communities at different depth over time. Perturbations in these patterns can impact primary productivity, geochemical cycling of nutrients, and ecosystem health. Detecting spatiotemporal patterns of the microbiome is an essential step to modeling and understanding microbial dynamics of an environment. In recent years, an increasing number of time-series studies of ocean microbiota have generated large datasets of ocean microbiome and environmental attributes to gain ecological insights into microbial community stability and dynamics over time (Gilbert et al., 2012; Giovannoni and Vergin, 2012). Studies using time-series analytical techniques and tools have already produced dynamic models of microbial communities, revealing their periodic patterns in response to environmental factors, and quantifying perturbations in community structure in response to irregularities (Faust et al., 2015). Future Red Sea metagenomic studies should create dynamic models of the microbiota, accounting for various environmental

factors to predict the distribution pattern of microbes and their genes in different niches. With the relative isolation of the Red Sea from world oceans, and its high year round temperatures, the Red Sea could function as an advanced model for studying the potential impact of climate change on future oceans.

The authors of this paper and their colleagues are currently conducting large-scale metagenomic studies of the Red Sea at KAUST. The primary objective is to monitor microbial diversity and function over time, with a focus on coastal regions and pelagic zones. The brine pools, sediments, and the coral reef microbiomes will also be closely examined for their bio-prospecting potential. These studies endeavor to reveal the significance of microbial genes (i.e., their metabolic pathways, adaptation mechanisms, symbiotic interactions, and the cycling of carbon and nutrients), the overall role of microbes in sustaining the Red Sea ecosystem, and their response to environmental pollution. The data collected from these studies, when combined and compared with future Red Sea microbiome data, could offer a uniquely sensitive marker of environmental changes and global health. This work could help produce a model of influential environmental variables on Red Sea microbiome composition enabling future contributions towards more complete environmental microbiome models (such as the Earth Microbiome Project). We are also exploring the Red Sea hot brine pools and sediments for novel enzymes including lipases, cellulases, and esterase for biotechnology-based applications. Function and sequence-based metagenomics, single cell genomics, synthetic biology, and metabolic engineering are amongst some of the many approaches used to screen and identify desired genes and metabolic pathways and express them in microbial cell factories for mass scale production.

As the omics (metagenomics, metatranscriptomics, metabolomics, and single cell genomics) datasets expand and include diverse marine environments, our ability to create dynamic models of marine microbiota increases. This would require large-scale multidisciplinary collaborations between microbial ecologist, microbiologists, bioinformaticians, and biostatisticians. Standardized methodologies and approaches are also needed to ensure successful and reproducible outcomes. The key points to consider for future metagenomic studies of the Red Sea are the establishments of “large-scale collaborations” and “standardized approaches” to “generate and share large datasets” for creating “dynamic models” of the Red Sea microbiota. The anticipated advances in NGS technologies and bioinformatics software and tools in the next decade would allow for more accurate interpretations of these large datasets, and more precise models.

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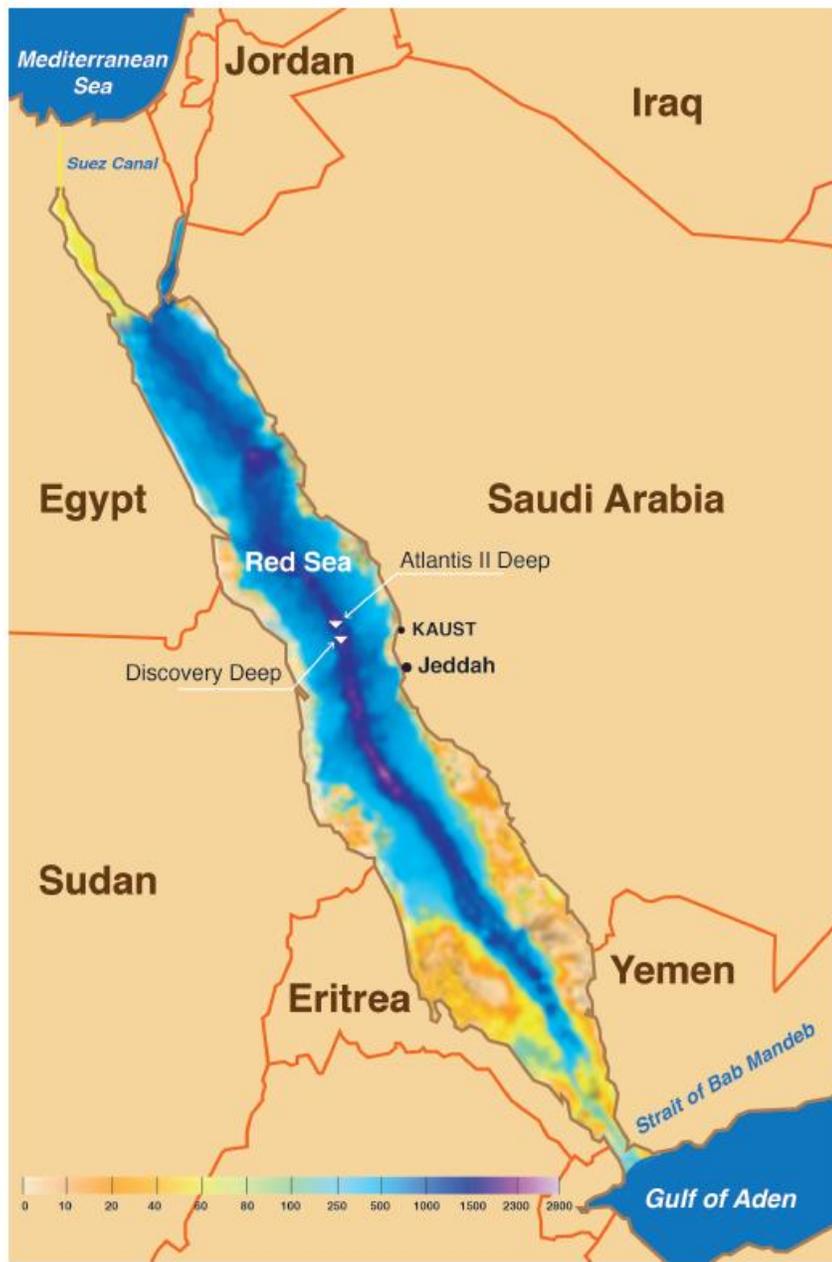
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**Figure Legend**

**Figure 1.** A map of Red Sea showing its location and position relative to the Mediterranean Sea in the North and the Gulf of Aden in the South. The color bar indicates the depth in meter (for the Red Sea only). White arrowheads show two representative brine pools, Atlantis II Deep and Discovery Deep.



**Abbreviations:**

UV, ultraviolet; NGS, next-generation sequencing; psu, practical salinity units; DO, dissolved oxygen; BSI, brine-seawater interface; OUT, Operational Taxonomic Unit; AOA, ammonia oxidizing Archaea; KAUST, King Abdullah University of Science and Technology

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