Biodiversity and 16S Ribosomal RNA Application in Space Biotechnology

Morteza Hanifezadeh[1], Reza Rezaii [2] ,Maryam Saadat Jalali Parvin*[2]

Abstract

Modern space biotechnology is a wide term which can cover a wide range of ways of changing the genetic material - the DNA code - in a living organism.

Preparing suit Microorganism needs complete information of earth microorganism's population. The comparison of rRNA sequences is a powerful tool for deducing phylogenetic and evolutionary relationships among bacteria, archaebacteria, and eucaryotic organisms. A set of oligonucleotide primers capable of initiating enzymatic amplification (PCR) on a phylogenetically and taxonomically wide range of bacteria is described along with methods for their use and examples. We name 16S Ribosomal RNA as The molecule" of space biotechnology.

Key words: 16S rRNA – diversity -ARDRA – Archia –Halophytes

Introduction

There are many hypotheses about human migration to other planets in near future. There are plenty of planets in milk galaxy and other part of space for human inhibition. [4, 25, 26] Microorganisms are the first earth inhibitors that prepare ground to other organism's life. [1, 7, 8, 9, 19, 24]

Mars is one of candidate planet for human inhibition. Mars must prepare for our inhibition. [10, 18, 21, 22] We had to help this process. Microorganisms play important rule in this topic as they various effects on biogeochemical cycles (BGC). [19, 24, 1]

There is much molecular method to study microbial diversity but little research done. [1, 14, 17, 20, 27] There is no complete data about our planet microorganism biodiversity and most of this population is unknown. [1, 8, 17] But it is possible founded many species that can play strategic role in human future. This needs the scientific research's to identify novel microorganism for our future goals. There are two important aims for this research's.
We know something about Mars ecosystem (table1). There are many candidates for this purpose but Archea are most important. Founding of new species is very important and has many advantages for us. Probably special species of microorganisms will found with our favorite characteristic in unbelievable ecosystems. Earth is variety of ecosystems that unfortunately not studied yet. [8, 11, 17] Space biotechnology is newest view for

biotechnology that will have great potential for effecting on human future well being, particularly about preparing new planet for human inhibition. [5, 6, 7, 10, 15, 18, 23]

The aim of this study is to fully understand the diversity complexity about the archaeal composition of Gurigol microbial mats and may provide insight in their contribution to the microbial mats and especially the role of methanogenesis, a process strictly performed by Archaea (Thauer, 1998). We applied massive parallel 16S rRNA gene tag sequencing (Sogin et al., 2006) allowing the analysis of several thousands of archaeal 16S rRNA gene fragments, which tremendously increased the resolution of assessing microbial diversity.This article will study advantage of Amplification of 16sRNA by PCR [3, 16, 20]

We are living on a planet with wide environmental changes. 99.9% of all specie that have existed is present. (Figure. 1)
Trustable and high speed molecular methods of microorganism's identification among eternity of ecosystems microorganism diversity are strategic tools for this purpose. [11, 3, 27, 20]

Materials and Methods

Sample Site and Sampling

Samples were taken from microbial mats developing on the North of Persian gulf (Figure 1). The samples were taken during three different seasons (autumn 2010, spring 2010 and summer 2010) from three different microbial mat types located along the tidal gradient in close proximity of each other (Table 1). Approximately 7-10mm of the top layer of the sediment containing the whole microbial mat was dissected, flash frozen in liquid nitrogen and stored at –80 1C.

Nucleic Acid Extraction

Total community DNA was isolated from 5 g (wet weight) of mat using the Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer’s instructions for maximal yields. DNA yields were determined using spectrophotometry (Table 1) and the quality was checked by PCR amplification of the archaeal 16S rRNA genes. Table 1 Description of sampling points

16S rRNA Gene Tag Sequencing

Tag sequencing was performed as previously described (Sogin et al., 2006; Huber et al., 2007). In short, conserved sequences flanking the hypervariable V6 region of rRNAs served as primer sites to generate PCR amplicons. In order to capture the full diversity of archaeal sequences, one 50 primer and two 30 primers were used (Table 2). PCR reaction was run in triplicate. The purified amplicon libraries were annealed to oligonucleotides that are complementary to an adaptor sequence that are tethered onto mm-size beads. The annealing conditions were set to favor one fragment per bead. PCR amplification of these fragments

generating B10 million identical copies of a unique DNA template per bead. Subsequently, the beads carrying the singlestranded
DNA clones were deposited into the wells of the PicoTiterPlate for pyrosequencing on a Roche Genome Sequencer (Roche Diagnostics GmbH/454 Life Sciences Corporation, Branford, CT, USA) (Sogin et al., 2006).

Sequence Read Analysis

Sequence reads of on average 60 bps were generated after trimming of the primer sequences from beginning and end of the raw data. Based on the assessment of pyrosequencing error rates, sequences of low quality were removed (Huse et al., 2007).

Alignment and taxonomy assignment was performed using the tag mapping methodology GAST (global alignment for sequence taxonomy) (Sogin et al., 2006; Huse et al., 2007). Sequence reads were compared with a reference database of known 16SrRNA genes (obtained from SILVA and Ribosomal Database Project (RDP) databases) and taxonomically assigned according to the RDP classifier (Wang et al., 2007).

Statistical Analysis

Taxonomic richness and diversity estimators were calculated using DOTUR/MOTHUR (www.mothur.org) software packages (Schloss and Handelsman, 2005; Schloss et al., 2009). We used the mean of the estimated richness for comparison between the samples. Cluster analysis of the community composition was performed using the statistical software package PAST (http://folk.uio.no/ohammer/past/) using a correlation matrix determined by the complement 1-r of Pearson's r correlation across the variables (Hammer et al., 2001).

Results
Description of the Sampling Sites

Station Hormozgān is one of the 30 provinces of Iran. It is in the south of the country, facing Oman. Its provincial capital is Bandar Abbas. The province has 14 islands located in the Persian Gulf, and 1,000 km (620 mi) of coastline. It is in the south of the country, facing Oman. Bandar Abbas has a hot and humid, but dry climate. Maximum temperature in summers can reach up to 49 °C (120 °F) while in winters the minimum temperature drops to about 5 °C (40 °F). The annual rainfall is around 251 mm (10 in) and the relative humidity is 66%.This area consist of small, young dunes, puddles of water and is densely vegetated with typical salt marsh plant species like Aerva persica, Calotropis procera, Heliotropium bacciferum, Anabasis setifera, Atriplex Leucoclada, Capparis decidua and Sphaerocoma ancheri. Microscopy revealed a high cyanobacterial diversity .(Severin and Stal, 2008; Dijkman et al., 2010). For the archaeal fraction we have a complete data set that showed a similar trend of highest richness and diversity.
Rarefaction analysis of the archaeal richness also did not yet reach an asymptote (Supplementary Figure S2) and the observed richness covered 74–80% of the estimated archaeal richness.

Cluster analysis at the phylum level revealed a conservation of the community composition in time in a specific mat type. The stations ST3 and ST1 were more similar and clustered separately from station ST2 (Figure 2).

A similar distribution was also found for the archaeal community; stations ST1 and ST3 clustered and their compositions were dissimilar from ST2.

Taxonomic Description

The archaeal diversity varied between the stations (Figure 3). A full description of the archaeal taxonomic data can be found in Supplementary Table S2. Station ST1 was dominated by Euryarchaeota (80–90%), mainly Methanobacteria or Methanomicrobia. The crenarchaeotal class Thermoprotei is only present in population of ST1. The population of ST1 contained an important fraction of NA Crenarchaeota and Euryarchaeota (13% and 10%, respectively). Station ST3 was dominated by Euryarchaeota of the class Halobacteria, where it accounted for 96% of the archaeal community. The community of ST3 contained Halobacteria, Methanomicrobia and a large fraction (29%) of NA Euryarchaeota. Station ST2 differs from the other two stations by having more NA Archaea, lower numbers of Euryarchaeota (42–56%) and more Crenarchaeota (11–39%). At the class level, the spring and summer communities of ST2 were dominated by Methanomicrobia. In ST2, Halobacteria were only present in low numbers.

Discussion

Richness analysis at a 95% cutoff value shows that it covered on average 75% (Archaea) of the expected richness based on the Chao1 estimator (Table 4), and thus our data set gives a good representation of the actual microbial diversity. The average Shannon diversity index (H) calculated for the coastal mat samples indicates a relatively high diversity in comparison with eight different marine environments that were previously described by Sogin et al (2006). At a 0.05 OUT cutoff, the average H index was estimated to be 5.2±0.3 for the eight marine environments (Schloss et al., 2009), whereas our samples revealed an average H index of 5.9±0.4. This confirms previous reports that coastal microbial mats are among the most diverse marine ecosystems (Allen et al., 2009).

The high diversity of microbial mats was attributed to the multitude of potential environmental niches and the large daily fluctuations of the key geochemical parameters (for example, pH, oxygen and sulfide) (Allen et al., 2009). Such conditions select for diverse microbial assemblages that on their turn drive diversity through niche creation, and hence forming a feedback between chemical complexity and biological diversity (Ley et al., 2006). The lower diversity in spring is in agreement with the succession of a young developing mat community that reaches maturity in summer and a climax during early autumn, after which the community becomes less productive, low in biomass and low in organic supply (Stal et al., 1985; Paerl et al., 1996).

The archaeal composition also clustered per station but did not reveal the grouping of ST1 with ST3. Apparently, factors driving the bacterial community composition were different from those that affected the archaeal community. Seasonal variations were less pronounced in the bacterial communities and only slightly affected clustering. In both ST1 and ST3, the spring and summer bacterial populations were more similar to each other than to the autumn

population. This is in agreement with general observations of seasonality of annual microbial mat communities.
The archaeal community composition in ST2 appeared to be stable, whereas the composition in ST1 and ST3 underwent large fluctuations. In ST1 the Methanobacteria dominated the community in autumn and was replaced in the following summer by Methanomicrobia. Long-term analysis over several consecutive years should indicate whether this was part of a yearly shift in methanogenic communities in response to mat maturation or whether this was a one-time event. For the ecosystem function this change has probably little impact as both are involved in methanogenesis. In ST3, the archaeal fraction was dominated by Halobacteria.
Notably in summer the observed richness was for 96% attributed to halophilic Archaea. These summer samples were taken after some weeks of high temperatures and low rainfall causing desiccation and increased local salinities, giving way for halophilic organisms. Sequences of a typical bacterial halophilic genus, Halobacillus, were slightly increased in the summer community of ST3, whereas the halotolerant Halochromatium was mainly found in the summer community of ST1. Salinibacter, although present in ST3, did not reveal higher numbers in the summer community.
Despite the harsher conditions during summer that favor growth of halophiles, the diversity in ST3 was only slightly lower (H¼5.8) than in autumn and spring (H¼6.1). Possibly, the non halotolerant microorganisms were protected from desiccation by the copious amounts of extracellular polymeric substances. Alternatively, the halophilic Archaea may have been confined to hypersaline microhabitats or salt crystals within the extracellular polymeric substance matrix formed during exsiccation (Norton and Grant, 1988)
Each coastal mat type also contained considerable numbers of sequence reads related to three candidate divisions
(TM7, WS3 and OD1) of which no cultivated representatives exist to date. Hitherto, no distinct ecological function has been assigned to members of the candidate division TM7 that are found in a wide range of environments, including terrestrial (soils, rhizosphere and peat bog) and aquatic (groundwater, fresh water, sea water, and deep-sea sediments) habitats (Rheims et al., 1996; Hugenholtz et al., 2001). Members of the candidate division WS3 members have been found associated with methanogenic environments (Dojka et al., 1998). The OD1 division is widespread in environments dominated by sulfur, such as marine environments and sulfureta (Harris et al., 2004).

Functional Diversity

Functional diversity was derived from those sequence reads that were assigned to a family, genus or higher taxonomic levels of which the functional properties are strongly conserved. For example, sequence reads assigned as Rivularia sp. are considered to be derived from dinitrogen-fixing photoautotrophes (Livingstone et al., 1984). Obviously, this might underestimate the actual number of contributors to ecosystem functions because potential functional groups may be hidden in sequences that could not be assigned beyond the taxonomic level of phyla, class or order.

Various Archaea like Methanosarcina were found in all stations and may also contribute to N2 fixation (Belay et al., 1984). Whether anaerobic ammonium oxidation (anammox) (Mulder et al., 1995) takes place in these mats remains uncertain as no sequence reads were found directly related to the known anammox species among the Planctomycetes. However,

the largest fraction of Planctomycetes (B2500 reads) could not be assigned below the family level and at this point we cannot exclude that it may contain a hitherto unknown genus involved in the anammox process.
Methanogenesis and methanotrophy are processes occurring in a wide variety of ecosystems. In sulfaterich marine sediments, methanogens are readily outcompeted by the SRB when competing for substrates such as acetate and hydrogen (Oremland et al., 1982). However, methanogens can use noncompetitive substrates such as methylamines, dimethylsulfoniopropionate and dimethylsulfide that are poorly used by SRB and are present in high concentrations in microbial mats (Oremland et al., 1982; Visscher et al., 1991; Jonkers et al., 1998). Methanogens all belong to the Archaea and considerable numbers of sequence reads related to methanogen sequences were present in all three stations, suggesting that methanogenesis is important in our mats. Methanogens related to Methanocorpusculum, Methanospirillum and Methanobacterium were restricted to ST1, whereas Methanomicrobiaceae were mostly found in station ST2. In ST1, Methanobacteria dominated the autumn community but were replaced by Methanomicrobia during the following summer. This change in gene pool would presumably not have affected ecosystem function with respect to methanogenesis.
Sequence reads assigned as originated from methanotrophic bacteria were present at very low numbers and presumably did not play an important role in methane consumption in the investigated microbial mats. Methane may rather be emitted from the mats into the air or may be oxidized anaerobically by methanogenic Archaea (for example, Methanosarcinales) via reversed methanogenesis in a tight consortium with SRB (Boetius et al., 2000). In summary, stabilization and fertilization of the bare sand by the dinitrogen-fixing microbial mats provides the prerequisites for vegetation to colonize sandy beaches. Not only did we confirm the major functional groups and geochemical processes in microbial mats that have been established before (Stal et al., 1985; van Gemerden, 1993; Severin et al., 2010), we also unveiled a previously unpredicted richness and diversity of Archaea.
Among the marine ecosystems studied by massive parallel 16S rRNA gene tag sequencing, the microbial mats are the most diverse. Previous studies suggested species richness in the order of B50 OTUs at 97% identity and B500 OTUs at 100% identity (Severin et al., 2010). Here we have found a more realistic estimator for bacterial richness of B3000 OTUs at 95% and B12 000 OTUs at 100% identity. At 100% identity, this would largely describe ‘within-species diversity’ resulting in ecotypes of species adapted to their particular microniches. This study also suggests that we have uncovered the tip of the iceberg with respect to biochemical potential and the possible complex reactions between the different members of Bacteria and Archaea of the mat, while a yet unknown microeukaryotic and viral diversity still needs to be explored.
Acknowledgements
We thank Mr.A.Barzggari and Dr.Aminfar from DANESHGAHE ELMI VA KARBORDI. We especially thank Dr. Sh.Dabirii Oskuii and Dr A.Dehnad for their support in laboratory and initial data analysis.

Future and Perspective

Microbial diversity is so important because of its impact on our future. New species infect main new metabolite, new characters, new drug and chance for new kind of human life.
Migration to other planet is one of the space plans. We need potential of new microorganisms to prepare other planet to human kind inhibition. However there is no complete information

about microorganism's diversity. Knowledge about microorganism diversity is an important for us to save human life in future. [18, 13, 10, 4, 1]
Space biotechnology with the base goal of gen engineering to make new spices of microorganism with favorite ability is our necessity. [6, 5, 10, 24, 23]
Although ARDRA couldn't differentiate between spices with less than 45% DNA homology, but ARDRA is a responsible, valid and completely specific technique. It is recognizing a genetic profile is peerless. [20, 27]
In conclusion we had to find new spices of microorganism sooner than what we can think to make our ideal microorganism by genetic engineering to gain space biotechnology.

References

1. A .T Bull, M Goodfellow, and J H Slater .Biodiversity as a Source of Innovation in Biotechnology Annual Review of Microbiology Vol. 46: 219-246 (Volume publication date October 1992) http://www.annualreviews.org/doi/abs/10.1146/annurev.mi.46.100192.001251

2. Adrian E. Radulovici, Philippe Archambault and France Dufresne .DNA Barcodes for Marine Biodiversity: Moving Fast Forward? March 2010 http://www.mdpi.com/1424-2818/2/4/450/

3. Adriana Giongo , Austin G. Davis-Richardson , David B. Crabb and Eric W. Triplett .Modifying Current 16S rRNA Databases for the Rapid Classification at Six Taxonomic Levels .June 2010 http://www.mdpi.com/1424-2818/2/7/1015/

4. André Bos. And el Tim Grant .Space Autonomy as Migration of Functionality: The Mars case. www.crewassistant.com/docs/pub/SMCIT06_TGrant_Paper.pdf

5. Augusto Cogoli .space biotechnology .page 215 Cell Biology and Biotechnology in Space, Volume 8 (Advances in Space Biology and Medicine) 2002 http://www.amazon.com/gp/product/0444507353/ref=sip_pdp_dp_0

6. Augusto Cogoli .biotechnological experiments - Page 217Cell Biology and Biotechnology in Space, Volume 8 (Advances in Space Biology and Medicine) 2002 http://www.amazon.com/gp/product/0444507353/ref=sip_pdp_dp_0

7. Berndt Feuerbacher , Heinz Stoewer.Utilization of Space: Today and Tomorrow .November 2005 http://www.amazon.com/Utilization-Space-Tomorrow-Berndt-Feuerbacher/dp/3540252002/ref=sid_dp_dp

8. Biodiversity http://en.wikipedia.org/wiki/Biodiversity

9. Ecology/Biogeochemical cycles http://en.wikibooks.org/wiki/Ecology/Biogeochemical_cycles

10. Fazale Rana .Bacteria or Boulders? Methane and Life on Mars. 8/31/2005 http://www.reasons.org/astronomy/life-other-planets/bacteria-or-boulders-methane-and-life-mars-0

11. Ferdinando Boero .The Study of Species in the Era of Biodiversity: A Tale of Stupidity Published: 22 January 2010 http://www.mdpi.com/1424-2818/2/1/115/

12. J. Jofre and A.R. Blanch Feasibility of methodes based on nucleic acid amplification techniques to fulfil the requrement for microbiological analysis of water quality. 2010 Journal of Applied Microbiology http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2010.04830.x/pdf

13. James M. Manyika, Roger P. Roberts, and Kara L. Sprague .Eight business technology trends to watch .Eight emerging trends are transforming many markets and businesses. Executives should learn to shape the outcome rather than just react to it.DECEMBER 2007 http://www.mckinseyquarterly.com/High_Tech/Strategy_Analysis/Eight_business_technology_trends_to_watch_2080?gp=1

14. Jennifer L. Kirka, Lee A. Beaudettea, Miranda Hartb, Peter Moutoglisc, John N. Klironomosb, Hung Leea, Jack T. Trevorsa .Methods of studying soil microbial diversity, Journal of Microbiological Methods 58 (2004) 169– 188

15. Jeanedy M. Pazinato , Ezio N. Paulo , Lucas W. Mendes , Rosana F. Vazoller and Siu M. Tsai .Molecular Characterization of the Archaeal Community in an Amazonian Wetland Soil and Culture-Dependent Isolation of Methanogenic Archaea .July 2010 http://www.mdpi.com/1424-2818/2/7/1026/

16. Jyoti Keswani‹ and William B. Whitman. Relationship of 16S rRNA sequence similarity to DNA hybridization in prokaryotes. International Journal of Systematic and Evolutionary Microbiology (2001), 51, 667–678

17. Halyan chu,Noah Flerer,christian L.Lauber, J.G.Caporaso, Rob Knight and paul Grogan .Soil bacterial diversity in Arctic is not fundamentally different from that found in other biomes 2010 http://www.colorado.edu/eeb/EEBprojects/FiererLab/Chu_etal_2010_arcticsoils.pdf

18. Howard Falcon-Lang .Will Mars be next? http://www.dominicantoday.com/dr/forum/living-in-the-dr/general-info/3638/Will-Mars-be-Next

19. Laurie Patsalides .Role of Bacteria in Marine Biogeochemical Reactions Feb 21, 2010.http://www.brighthub.com/environment/science-environmental/articles/64561.aspx

20. Lentil Ufuk CELIKKOL AKCAY,Gulsum KALEMTAS,Meral YUCEL,Huseyin Avni OKTEM .Comparison Of Different Molecular Methods In Screening Genetically Modified Journal of Engineering Science and Design Vol:1 No:2 pp.73-78, 2010 http://edergi.sdu.edu.tr/index.php/mbtd/article/viewFile/1668/1884

21. Life in Space for Life on Earth 2010. June 2010, Italy http://www.congrex.nl/10a09/

22. Mars http://en.wikipedia.org/wiki/Mars

23. NASA to Showcase Space Biotechnology Source: NASA HQ 2003 http://www.spaceref.com/news/viewpr.html?pid=11856

24. Rebecca Boyle.Future Mars Colonists Could Learn To Terraform By Studying Darwin's Methods .2010 http://www.popsci.com/science/article/2010-09/future-mars-colonists-could-learn-terraform-studying-darwins-methods

25. Robert D. Twitchell Stephen Horne.Predicting the future of humans inhabiting other planets. http://www.helium.com/items/139827-predicting-the-future-of-humans-inhabiting-other-planets
26. Tracy R. Holcombe, Thomas J. Stohlgren and Catherine S. Jarnevich .From Points to Forecasts: Predicting Invasive Species Habitat Suitability in the Near Term. May 2010 http://www.mdpi.com/1424-2818/2/5/738/

27. Vieites JM, Guazzaroni ME, Beloqui A, Golyshin PN, Ferrer M.Molecular methods to study complex microbial communities . http://www.ncbi.nlm.nih.gov/pubmed/20830553

28. William G. Weisburg, Susan M. Barns, Dale A. Pelletier, and David J. Lane .16S Ribosomal DNA Amplification for Phylogenetic Study. journal of bacteriology, Jan. 1991, p. 697-703 Vol. 173, No. 2

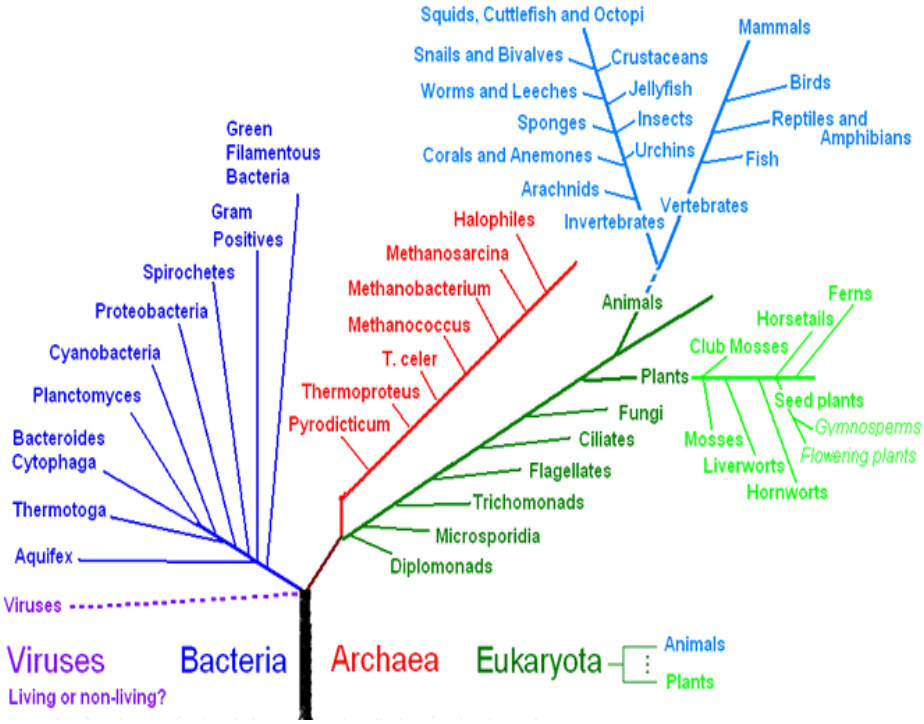
Physical characteristics			
Mean density	3.9335 ± 0.0004 g/cm³		
Surface temp.	min	mean	max
	186 K	210 K	293 K
Kelvin	−87 °C	−63 °C	20 °C
Celsius			

Atmosphere	
Surface pressure	0.636 (0.4–0.87) kPa
Composition	95.32% carbon dioxide 2.7% nitrogen 1.6% argon 0.13% oxygen 0.08% carbon monoxide 210 ppm water vapor 100 ppm nitric oxide 15 ppm molecular hydrogen 2.5 ppm Neon 850 ppb HDO 300 ppb Krypton 130 ppb formaldehyde 80 ppb xenon 30 ppb ozone[*citation needed*] 18 ppb hydrogen peroxide 10 ppb methane

Tabale.1. Mars's ecosystem

Fig.1.Biodiversity is the variation of life forms within a given ecosystem. It is shown as a life tree

Figure 1 Aerial picture and geographical location of the sampling stations (see insert) at the northwestern part of the North Sea coast of
the Dutch barrier island Schiermonnikoog. Coordinates of the sampling points are presented in Table 1. See Materials and methods for a description of the stations. The pictures were obtained with Google Maps.




Lesser Tunb
Abu Musa
Greater Tunb
Hormozgan
Qushm Island
Bandar-e-Abbas
Sample 3
Sample 4
Sample 1
Ras Al-Khaimah
© 2011 Google
© 2011 Cnes/Spot Image
© 2011 LeadDog Consulting
© 2011 Europa Technologies
27°14'22.51" N 56°14'00.24" E elev. 392 m
Alt. ojo 366.14 km
Google