Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2019, 8 (5): 2051-2058

(International Peer Reviewed Journal)

Secondary Structural Analysis of rRNA Sequences of Hydrogen Producing Purple non Sulphur Phototrophic Bacteria

Swati Mohapatra[1], Sunil Kumar Sharma[2] and Ramchander Merugu[3]*

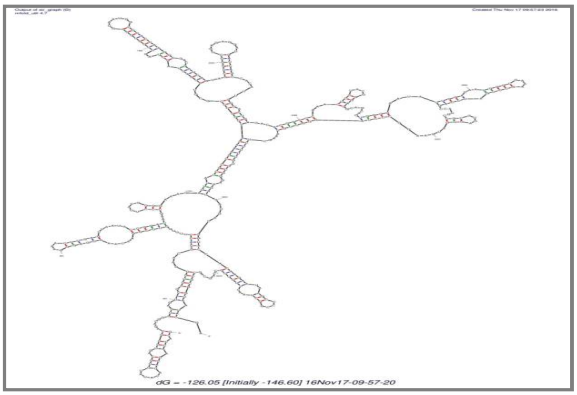
1. Department of Biotechnology, IIT Roorkee, Uttarakhand- 247667, **INDIA**
2. Department of Mechanical Engineering, Amity University, Noida, New Delhi- 201313, **INDIA**
3. Department of Biochemistry, Mahatma Gandhi University, Nalgonda, Telangana-508254, **INDIA**

Email: rajumerugu01@gmail.com

ABSTRACT

In the present investigation, genetic variability has been observed at RNA secondary structures, indicating evolution apparently acting at structural levels of RNA. It has also been observed that the rRNA folding pattern in KF011915 Rhodobacter sphaeroides SMR001 is the most stable energetically (i.e.-425.40 kcal mol^{-1}) compared to other Rhodobacter sphaeroides strain. On the contrary, the r-RNA folding energy of KM189155 Rhodopseudomonas faecalis MPPR 001 is -146.60 kcal mol^{-1} and KJ881378 Rhodopseudomonas palustris strain SMR001 (310.00 kcal/mol) is more stable among all the Rhodopseudomonas species. The 16S ribosomal divergence has been carried in order to understand its variability

Graphical Abstract



Keywords: Secondary structural analysis, rRNA sequences, Purple non sulphur phototrophic bacteria, Hydrogen.

INTRODUCTION

The most widely accepted method for molecular identification is 16S rRNA gene sequencing [**1**]. The 16S rDNA also plays a significant role in molecular identification due to the conserved and variable regions within the gene [**2**]. Compared to it the 16S rRNA secondary structure of the gene is more conserved, hence it is used in the phylogenetic analysis [**3**]. The prime objective of the present study

was to compare the secondary structures of 16S rRNA gene sequences of the hydrogen producing purple non sulphur phototrophic bacteria. The stability of rRNA sequences was investigated from different species of phototrophic bacteria. The organisms which were involved included species from *Rhodobacter* and *Rhodospeudomonas*, both of which are the most widespread organisms in the group of purple non sulphur bacteria. Both have been widely investigated for the production of biohydrogen. The sequences of the hydrogen producing bacteria were downloaded from the NCBI website and are presented below.

>KT824856 *Rhodopseudomonaspalustris* SMR006:
TAAACGATGAATGCCAGCCGTTAGTGGGTTTACTCACTAGTGGCGCAGCTAACGCTTTAA
GCATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGACGCAACGCGCAGAACCTTACCAGCCCT
TGACATGTCCAGGACCGGTCGCAGAGATGTGACCTTCTCTTCGGAGCCTGGAGCACAGGT
GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCCCGTCCTTAGTTGCTACCATTTAGTTGAGCACTCTAAGGAGACTGCCGGTGATAA
GCCGCGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACAC
ACGTGCTACAATGGCGGTGACAATGGGATGCTAAGGGGCGACCCCTCGCAAATCTCAAA
AAGCCGTCTCAGTTCGGATTGGGCTCTGCAACTCGAGCCCATGAAGTTGGAATCGCTAGT
AATCGTGGATCAGCA

>KT824855 *Rhodopseudomonaspalustris* SMR005:
CGGGCGTAGCAATACGTCAGTGGCAGACGGGTGAGTAACGCGTGGGAACGTACCTTTTG
GTTCGGAACAACTGAGGGAAACTTCAGCTAATACCGGATAAGCCCTTACGGGGAAAGAT
TTATCGCCGAAAGATCGGCCCGCGTCTGATTAGCTAGTTGGTGGGGTAATGGCCCACCAA
GGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGC
CCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCC
AGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTTGTGCGGGAAGA
TAATGACGGTACCGCAAGAATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATA
CGAAGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGTGCGTAGGCGGGTTTCT
AAGTCAGAGGTGAAAGCCTGGAGCTCAACTCCAGAACTGCCTTTGATACTGGAAGTCTT
GAGTATGGCAGAGGTGAGTGGAACTGCGAGTGTAGAGGTGAAATTCGTAGATATTCGCA
AGAACACCAGTGGCGAAGGCGGCTCACTGGGCCATTACTGACGCTGAGGCACGAAAGCG
TG

>KT824852 *Rhodopseudomonaspalustris*SMR002:
TACGTCAGTGGCAGACGGGTGAGTAACGCGTGGGAACGTACCTTTTGGTTCGGAACAAC
TGAGGGAAACTTCAGCTAATACCGGATAAGCCCTTACGGGGAAAGATTTATCGCCGAAA
GATCGGCCCGCGTCTGATTAGCTAGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCA
GTAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGC
GTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTTGTGCGGGAAGATAATGACGGTA
CCGCAAGAATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCT
AGCGTTGCTCGGAATCACTGGGCGTAAAGGGTGCGTAGGCGGGTTTCTAAGTCAGAGGT
GAAAGCCTGGAGCTCAACTCCAGAACTGCCTTTGATACTGGAAGTCTTGAGTATGGCAG
AGGTGAGTGGAACTGCGAGTGTAGAGGTGAAATTCGTAGATATTCGCAAGAACACCAGT
GGCGA

>KJ881378.1 *Rhodopseudomonas palustris*SMR00:
CGGGGGCATACGTCAGTGGCGACGGGTGAGTACGCGTGGGAACGTACCTTTTGGTTCGG
AACAACACAGGGAAACTTGTGCTAATACCGGATAAGCCCTTACGGGGAAAGATTTATCG
CCGAAAGATCGGCCCGCGTCTGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGA
CGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAA
CTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCA

TGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTTGTGCGGGAAGATAATG
ACGGTACCGCAAGAATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAG
GGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGTGCGTAGGCGGGTTTCTAAGTC
AGAGGTGAAAGCCTGGAGCTCAACTCCAGAACTGCCTTTGATACTGGAAGTCTTGAGTAT
GGCAGAGGTGAGTGGAACTGCGAGTGTAGAGGTGAAATTCGTAGATATTCGCAAGAACA
CCAGTGGCGAAAGCGGCTCACTGGGCCATTACTGACGCTGAGGCACGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGATAGTCCACGCCGTAACGATGAATGCCAGCCGTTAGT
GGTTTACTCACTAGTGGCGCAGCTAACGCTTTAAGCATTCCGCCTGGGGAGTACGGTCGC
AGATTAAAACTCAAAGGAATTGACGGGGCCCGCACAAGC

>KM189155*Rhodopseudomonas faecalis* MPPR 001:
GCACCAAGCATACATACGTGCAGTGGGCAGACGGGTGAGTACGCGTGGGAACGTACCTT
TTGGTTCGGAACAACTGAGGGAAACTTGAGCTAATACCGGATAAGCCCTTACGGGGAAA
GATTTATCGCCGAAAGATCGGCCCGCGTCTGATTAGCTAGTTGGTGGGGTAATGGCCCAC
CAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACAC
GGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGA
TCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTTGTGCGGGAA
GATAATGACGGTACCACAAGAATAAGCCCCGGCTAACTTCTTGCCAGCAGCCGCGGTAA
TACGA

>KJ873881 *Rhodopseudomonasfaecalis* SMRJVI:
CGCCCTGGTCTTCATTGCCGACGGGTGCCTGCGCGTGGGGACGTACCTTTTGGTTCCTGC
CTCTGACGGGGGTTCACCCGATACCGGATAAACCCTTACGGGGAACGATTTATCGCCAAT
GATATATATTTGTCTGAATTGCTCGTTGGTGGATTGATGTGCCTTCCATTAGATTGTTGGT
GATGGTCTGAGAGGATGATCAGCCATGGATAGGGGAACTGAGGCCCAGACCCCCCCCGG
AGGCACTGATGGGGAATATTGACTCCTGGGGGAGGGCCTGATCCGGACATGTGGCCAAT
GGGATAAAGGCCCAACGGTTGTAAAGCTCTTTTGGACGAGAAGATTATGGCTTGTCCACT
TCTTTTGTCCCGGAATAACTTCTCGCCAGCACCCGCGGTAATACGAAGGGGGCTAGCGTT
GCTCGGAATCACTGGGCGCCAAGGGTGCGTATAATACGTTTCTAACCCAGAGTTGAAAG
GATGGTTGGGTCTCCAGAACTGCCTTTGATACTGGAAATTTTGAGGTCAAAAGAGGTGAG
TG

>KJ131189*Rhodopseudomonasfaecalis* SMR001:
ACCCTTTTTCGTGGCGACGGGTGAGTACGCGTGGGACGTACCTTTTGGTTCGGACAACTG
AGGGGAACTTCCCTAATACCGGATAAGCCCTTACGGGGAAAGATTTATCGCCGAAAGAT
CGGCCCGCGTCTGATTAGCTAGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCAGTA
GCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGG
GAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTG
AGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTTGGGCGGGAAGATAATGACGGTACCC
CTTGAATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGC
GTTGCTCGGAATCACTGGGCGTAAAGGGTGCGTATGCGGGTTTCTAAGTCAGAGGTGAA
AGCCTGGAGCTCAACTCCAGAACTGCCTTTGATACTGGAAGTCTTGAGTTCAGGAGAGGT
GAGTGGAACTGCTAGTGTAGAGGTGAAATTCGTAGATATTCGCAAGAACACCAGTGGCG
AAGGCGGCTCACTGGCCCGATACTGACGCTGAGGCACAAAAGCGTGGGGAGCAAACAG
GATTAGATACCCCTGGTAGTCCTCTCGTAAACGATGAATGCCAGCCCGTTCGTGGGTTTT
ACTCACTCGTGGCGCACCTAACCCTTTTAAGCATTCCCGCCTGGGGAGTACTGGTCTCAA
GATTAAAACTCAAAGGAATCTGTACGGGGGCTCCCCCAAGCCGTGGAAGCCT

>KF011915 *Rhodobactersphaeroides* SMR001:
CCTAAAACATGCAGTCGAGCGAGGACTTCGGTCCTAGCGGCGGACGGGTGAGTAACGCG
TGGGAACGTGCCCTTTGCTTCGGAATAGCCCTGGGAAACTGGGAGTAATACCGAATGTG
CCCTACGGGGGAAAGATTTATCGGCAAAGGATCGGCCCGCGTTGGATTAGGTAGTTGGT
GGGGTAATGGCCTACCAAGCCGACGATCCATAGCTGGTTTGAGAGGATGATCAGCCACA

CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTAGACAA
TGGGCGCAAGCCTGATCTAGCCATGCCGCGTGAGCGATGAAGGCCTTAGGGTTGTAAAG
CTCTTTCGTGGGGGAAGATAATGACTGTACCCCAAGAAGAAGCCCCGGCTAACTCCGTG
CCAGCAGCCGCGGTAATACGGAGGGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGC
GCACGTAGGCGGACTGGAAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCC
TTTGAAACTCCCAGTCTTGAGGTCGAGAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGA
AATTCGTAGATATTCGGAGGAACACCAGTGGCGAACGCGGCTCACTGGCTCGATACTGA
CGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGAATGCCAGACGTCAGCAGGCATGCCTGTCGGTGTCACACCTAACGAGATT
AAGCATTCCGCCTGGGGAGTACGGCCGCAAGGTTAAACTCAAAGGAATTTGACGGGGGC
CCGCACAAGGCGGTAGGAGCATGTGGTTTAATTCCGAAGCAACGCCGCAAGGAGCCTTC
ACCCACCCTTGGACATGGGTATTCGGCGGGACCAGAGATGGTCCCTTCAGATTCCGCTAG
CATACCCACCACAGTGCTGCATGGCTGTCAGTCAGCTCGGTTGTCGTGGAGAATGTTCGA
TTAAGTACGGCAACGAGCGCATCCCACACTTCAGTGCATCATTCAGTTGGGCACTC

>KT824854 *Rhodopseudomonaspalustris* SMR004:
TAACGCGTGGGAACGTACCTTTTGGTTCGGAACAACTGAGGGAAACTTCAGCTAATACC
GGATAAGCCCTTACGGGGAAAGATTTATCGCCGAAAGATCGGCCCGCGTCTGATTAGCT
AGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATC
AGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATAT
TGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCCCTAGGGT
TGTAAAGCTCTTTTGTGCGGGAAGATAATGACGGTACCGCAAGAATAAGCCCCGGCTAA
CTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCTCGGAATCACTGGGC
GTAAAGGGTGCGTAGGCGGGTTTCTAAGTCAGAGGTGAAAGCCTGGAGCTCAACTCCAG
AACTGCCTTTGATACTGGAAGTCTTGAGTATGGCAGAGGTGAGTGGAACTGCGAGTGTA
GAGGTGAAATTCGTAGATATTCGCAAGAACACCAGTGGCGAAGGCGGCTCACTGGGCCA
TTACTGACGCTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
CACGCCGTAAAC

>KT824853*Rhodopseudomonaspalustris*SMR003:
TACGTCAGTGGCAGACGGGTGAGTAACGCGTGGGAACGTACCTTTTGGTTCGGAACAAC
TGAGGGAAACTTCAGCTAATACCGGATAAGTCCTTACGGGGAAAGATTTATCGCCGAAA
GATCGGCCCGCGTCTGATTAGCTAGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCA
GTAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGC
GTGAGTGATGAAGGCCCTAGGGTTGTAAAGCTCTTTTGTGCGGGAAGATAATGACGGTA
CCGCAAGAATAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCT
AGCGTTGCTCGGAATCACTGGGCGTAAAGGGTGCGTAGGCGGGTTTCTAAGTCAGAGGT
GAAAGCCTGGAGCTCAACTCCAGAACTGCCTTTGATACTGGAAGTCTTGAGTATGGCAG
AGGTGAGTGGAACTGCGAGTGTA

MATERIALS AND METHODS

Phylogenetic analysis: Phylogenetic analysis of genes has served as a useful technique to study evolutionary relationships among different bacteria and virus population [**4**]. Nucleotide sequences of primarily identified hydrogen producing phototrophic bacteria were collected from the National Center for Biotechnology Information (NCBI) web server to perform a sequence analysis. Multiple Sequence Alignments (MSAs) and construction of a phylogenetic tree among these sequences were performed using the maximum likehood algorithm **[5]** in MEGA 6.0 (Molecular Genetics Evolutionary Analysis) package [**6**].

RNA secondary structure prediction: 16S rRNA of each bacterial sequence was predicted in the Mfold web server [**7**] to study and compare folding patterns among them. The minimum Gibb's free

energy, ΔG, was computed by the mfold algorithm for each sequence, as the lowest Δ*G* maps to evolutionary stability of RNA structures for ten hydrogen producing bacterial sequences. The temperature was fixed to 37°C. RNA sequences were taken as linear; the ionic conditions were fixed at [Na+] = 1 M and [Mg++M] = 0. These were compared according to methods discussed earlier [**8**].

RESULTS AND DISCUSSION

In silico molecular phylogenetic analysis and study of rRNA folding patterns along with phenotypic characterization of 16S rRNA genes [**8-10**] have served together as a more useful method for identification of bacteria than when these techniques are used alone. Characterization of bacteria isolated from the different ecological niches shows the dominance of gram-negative purple non sulphur bacteria which have the ability to produce hydrogen. The evolutionary relationship has been studied through a molecular phylogenetic approach (Figure 1), which revealed a strong closeness among hydrogen producing isolates of various ecological niches.

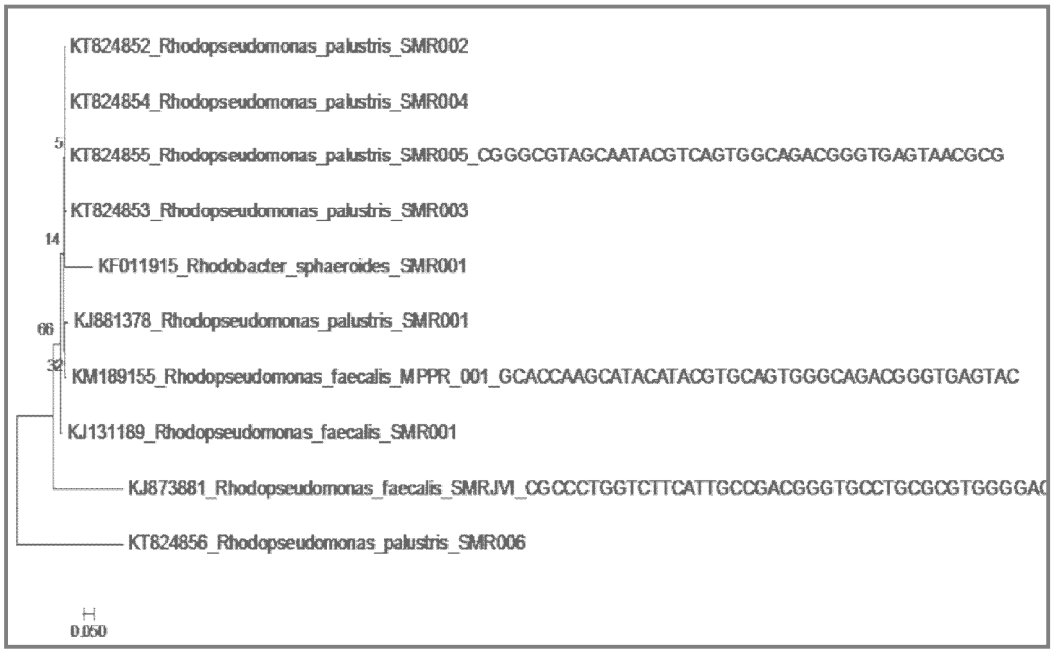
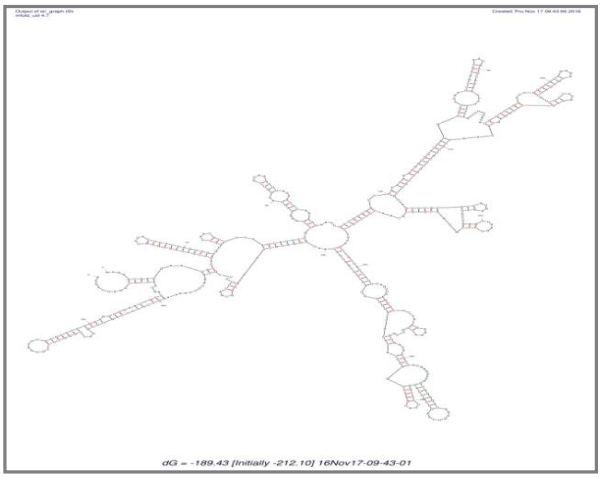


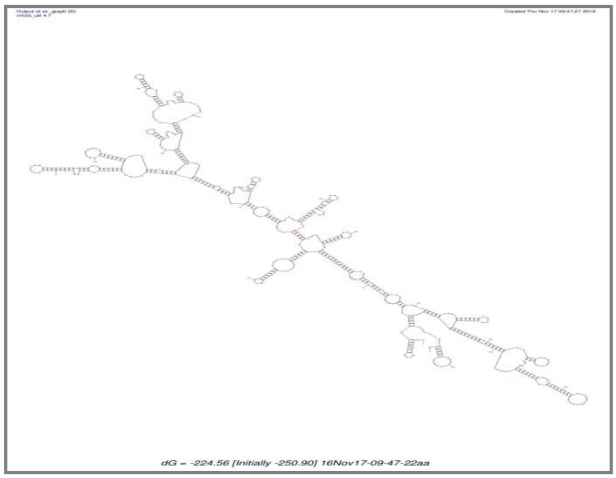
Figure 1. Phylogenetic analysis of the species.

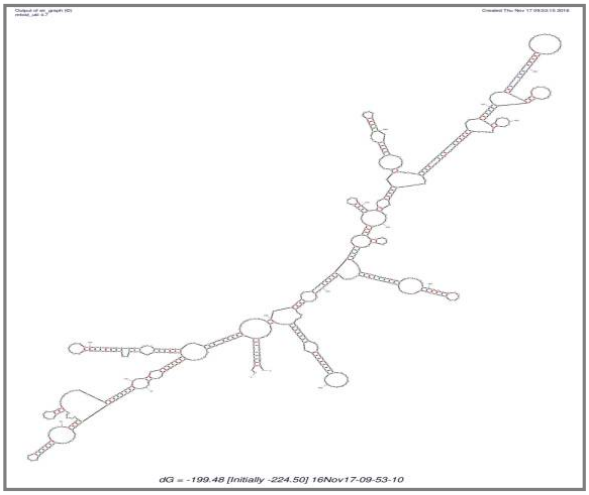
The secondary structure of a 16S rRNA is more conserved than its counterpart DNA sequence. Thus, in the present investigation, genetic variability has been observed at RNA secondary structures, indicating evolution apparently acting at structural levels of RNA. It has also been observed that the r-RNA folding pattern in KF011915 *Rhodobacter sphaeroides* SMR001 is the most stable energetically (i.e.-425.40 kcal mol^{-1}) compared to other *Rhodobacter sphaeroides* strain. On the contrary, the r-RNA folding energy of KM189155 *Rhodopseudomonas faecalis* MPPR 001 is -146.60 kcal/mol and KJ881378 *Rhodopseudomonas palustris* strain SMR001 (310.00 kcal mol^{-1}) is more stable among all the Rhodopseudomonas species (Table 1 and Figure 2). The 16S ribosomal divergence has been carried in order to understand its variability.The secondary structure of RNA has been predicted using MFold (Web address) software in order to study the 16s rRNA conserved pattern at the structural level. Two different bacterial genus strains have been analyzed for phylogenetic analysis by looking into their 16s r-RNA structural divergence. Bacterial strain 1, 5 and 6 showed the similar structural pattern as compared to other 10 strains whereas strain 2, 3, 7 and 9 share a common folding pattern. Strain 4, 8 and 10 have the same type of branching i.e. stem and the loop may belong to the same group.

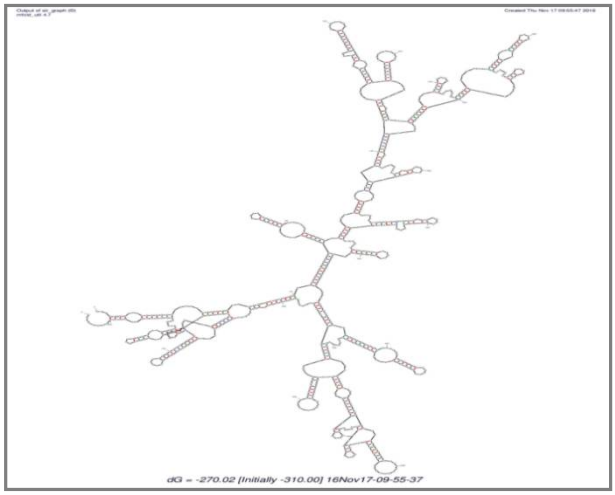
Table 1. ΔG values of the rRNA folding patterns of the species

1	ΔG = -212.10 kcal/mol, (KT824856 Rhodopseudomonaspalustris strain SMR006)
2	ΔG = -250.90 kcal/mol (KT824855 Rhodopseudomonaspalustris strain SMR005)
3	ΔG = -224.50 kcal/mol, (KT824852 Rhodopseudomonaspalustris strain SMR002)
4	ΔG = -310.00 kcal/mol, (KJ881378 Rhodopseudomonaspalustris strain SMR001)
5	ΔG = -146.60 kcal/mol, (KM189155 Rhodopseudomonasfaecalis strain MPPR 001)
6	ΔG = -197.30 kcal/mol (KJ873881Rhodopseudomonasfaecalis strain SMRJVI)
7	ΔG = -309.70 kcal/mol, (KJ131189 Rhodopseudomonasfaecalis strain SMR001)
8	ΔG = -425.40 kcal/mol, (KF011915 Rhodobactersphaeroides strain SMR001)
9	ΔG = -256.50 kcal/mol, (KT824854 Rhodopseudomonaspalustris strain SMR004)
10	ΔG = -204.60 kcal/mol, (KT824853 Rhodopseudomonaspalustris strain SMR003)









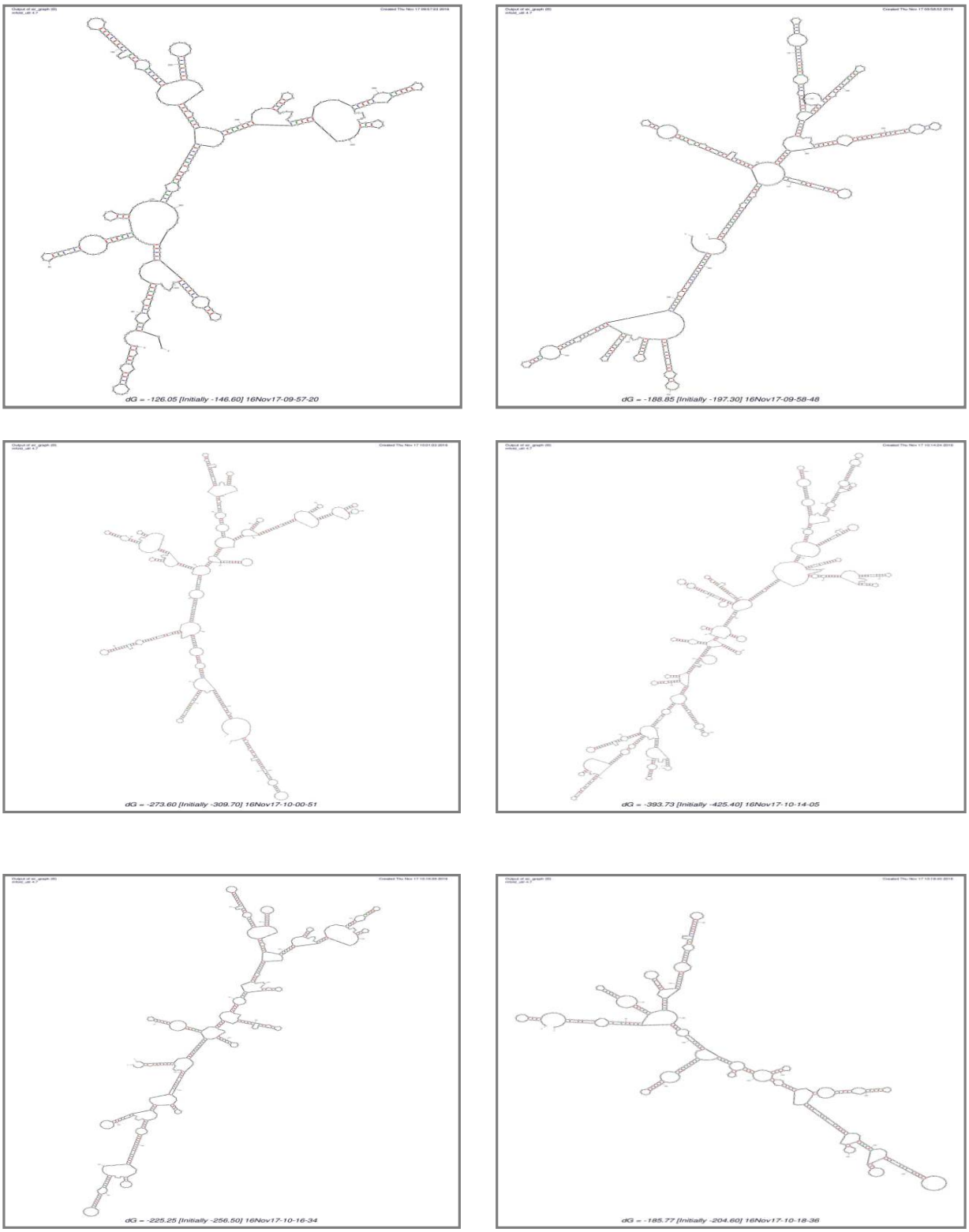


Figure 2. Secondary structure prediction of the 16srRNA.

APPLICATION

Earlier studies have correlated the relation between the rRNA folding patterns and metabolite production from other bacteria. Similarly this study can be applied for knowing whether a correlation can be found between the secondary structure of rRNA and the rates of hydrogen production.

CONCLUSION

Two different hydrogen producing bacterial genus strains have been analyzed for phylogenetic analysis by looking into their 16s r-RNA structural divergence. Bacterial strain 1, 5 and 6 showed the similar structural pattern as compared to other 10 strains whereas strain 2, 3, 7 and 9 share a common folding pattern. Strain 4, 8 and 10 have the same type of branching i.e. stem and the loop may belong to the same group. Based on the experimental rates of hydrogen production further studies of any correlation between hydrogen producing ability and rRNA folding can be made.

REFERENCES

[1]. A. R. Contreras., M. Koller, M. M. D. Dias, M. Calafell-Monfort, G. Braunegg, M. S. Marques-Calvo, High Production of Poly [3-hydroxybutyrate] from a Wild *Bacillus megaterium*Bolivian Strain, *J of Applied Microbiology*, **2013**, 114(5), 1378-1387.

[2]. J. M. Janda, S. L. Abbott, 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls, *Journal of Clinical Microbiology*, **2007**, 45(9), 2761-2764.

[3]. K. Bhattacharjee, S. Banerjee, S. R. Joshi, Diversity of *Streptomyces* spp. in Eastern Himalayan Region-computational RNomics Approach to Phylogeny, *Bioinformation*, **2012**, 8(12), 548-554.

[4]. S. Ambhore, S. Galande, L. Jena, S. Kumar, Phylogenetic Analysis of H1N1 Proteins for Understanding its Allocation, *Int J Bioautomation*, **2015**, 19(4), 311-324.

[5]. K. R. Shah, FTIR Analysis of Polyhydroxyalkanoates by Novel *Bacillus Sp.* AS 32 from Soil of Kadi Region, North Gujarat, India, *J of BiochemTechnol.*, **2012**, 3(4), 380-383.

[6]. K.Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0, *Mol Biol Evo*l., **2013**, 30, 2725-2729.

[7]. M. Zuker, Mfold Web Server for Nucleic Folding and Hybridization Prediction, Nucleic Acid Research, **2003**, 31(13), 3406-3415.

[8]. S. Mohapatra, D. P. Samantaray, S. M. Samantaray, Phylogenetic Heterogeneity of the Rhizospheric Soil Bacterial Isolates Producing PHAs Revealed by Comparative Analysis of 16s-rRNA, *Int J of Current Microbiol and ApplSci*, **2014**, 3(5), 680-690.

[9]. J. E. Clarridge 3rd, Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases, *Clinical Microbiology Reviews*, **2004**, 17(4), 840-862.

[10]. Jamal S.M. Sabir, Salah E.M. Abo-Aba, Ayman Sabry, Refaei M. Hussein, Ahmed Bahieldin and Nabeeh A. Baeshen, Isolation, identification and comparative analysis of 16S rRNA of Bacillus subtilis grown around Rhazya stricta roots, *Life Science Journal*, **2013**, 10(12), 980-986.