International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 9 | Special Issue 2 | 2019 | 294-302
| Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

Biophysical Characterization and Sequence Analysis of Cytochrome Oxidase in *Sargassum Muticum*

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Received: 30 Jan 2019 / Accepted: 20 Feb 2019 / Published online: 01 Apr 2019
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Abstract

Seaweed or benthic marine algae are the group of plants that live either in marine or brackish water environment. Like the land plants seaweed also contain photosynthetic pigments and with the help of sunlight and nutrient present in the seawater they photosynthesize and produce food. Green algae are found in the fresh and marine habitats. Brown algae are exclusively marine forms. The seaweed *Sargassum muticum* contain protein known as cytochrome oxidase. In eukaryotes the phosphorilating electron transfer chain is located in the inner membrane of their mitochondria and uses oxygen as the terminal sink for electron. The sequence of cytochrome oxidase retrieved from National Centre for Biotechnology information in fasta format. The structural homology of cytochrome oxidase was carried out by using Bioinformatics tools like protscale, propsearch, signal protein, target protein and sequence analysis were done through tools like codon plot, Anchor and Annie tool. Further studies are required to investigate the cytochrome oxidase of for potential pharmacological properties.

Keywords
Biophysical, Cytochrome Oxidase.

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INTRODUCTION

Seaweed or benthic marine algae are the group of plants that live either in marine or brackish water environment. Like the land plants seaweed contains photosynthetic pigments and with the help of sunlight and nutrient present in the seawater, they photosynthesize and produce food. Seaweeds are found in the coastal region between high tide to low tide and in the sub-tidal region up to a depth where 0.01 % photosynthetic light is available. Plant pigments, light, exposure, depth, temperature, tides and the shore characteristics combine to create different environment that determine the distribution and variety among seaweeds.

They are basically classified according to colour into three main groups

1. Green (Chlorophyta)
2. Brown (Phaeophyta)
3. Red (Rhodophyta)

Sargassum muticum is brown seaweed, normally brown to yellowish with a length up to 10 m. It is an autotroph that uses energy from sunlight. The photosynthesis is facilitated thanks to aerial a vesicle which allows the algae to rise to the surface.

Sargassum muticum is composed of two distinct parts: a perennial part, which contains the holdfast and one or more short main axes; and an annual part: the secondary axes, which develop on the main axis, whose growth is unlimited, and the size is variable. There are three types of ramifications: laterals with foliaceous expansions called fronds, laterals with fronds and aerocysts and laterals with fronds, aerocysts and reproductive organs called receptacles. In winter, only the perennial part persists (5 cm). In summer, the lateral part is in maximum development of 2–3 meters to 10 meters.

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Sargassum muticum is rich in antioxidant compounds such as phenolic compounds (cathechins, phlorotannins, quercetins), pigments (fucoxanthin) and vitamins (vitamin C, K, E in the form of alpha-tocophérol and gamma-tocopherol). Applications are possible in pharmaceutics, cosmetics and health fields for the antioxidant activities of these molecules.

METHODOLOGY

- Target proteins of cytochrome oxidase sequence were retrieved from NCBI database.
- The retrieved sequence is submitted to the following server and tool, for protein profiling and functional annotation.
- The retrieved sequence is submitted to the protscale tool, for the identification of primary sequence analysis in cytochrome oxidase.
- The retrieved sequence is submitted to the signalP tool, for the identification of Signal peptides in cytochrome oxidase.
- The retrieved sequence is submitted to the targetP tool, for the identification of transmembrane region s in cytochrome oxidase.
- The retrieved sequence is submitted to the codon plot tool, for the identification of coding region in cytochrome oxidase.
- The retrieved sequence is submitted to the DNA molecular weight tool, for the identification of cytochrome oxidase molecular weight.
- The retrieved sequence is submitted to the ORF finder tool, for the identification of ORF finder to know the open frame in cytochrome oxidase.
- The retrieved sequence is submitted to the ANCHOR tool, for the identification of protein binding region in cytochrome oxidase.
- The retrieved sequence is submitted to the ANNIE tool, for the identification of motif in cytochrome oxidase.

RESULT AND DISCUSSION

Sequence Retrieval -NCBI

Protein Sequence

>BAT47064.1 cytochrome oxidase subunit 1, partial (mitochondrion) [*Sargassum muticum*]
GFGNWFVPLMIGAPDMAFPRMNNISFWLLPPSLMLLLAS
SLVEAGAGTGWTVYPPLSGIQAHSGPSDLAIFSLHLSGAASI
LGAINFITTIFNMRAPGMGMHRLPLFVWSVLITAFLLLLSLP
VLAGGITMLLTDRNFNTTFFDPAGGGDPVLYQHLFWFFGH
PEVYILILPGFGIVSHILATFSRKPVFGYLGMVYAMLSIGILGFI
VWAHHMFTVGLDIDTRAYFTAATMIIAVPTGIKIFSWIATM
WGGSIRLKTPMLFSIGFLFLFTIGGLTGVVLANSGVDIALHD
TYYVVAHFHYVLSMGAAFTMFGAFYFWVGKMTGLGYPEV
LGQIHFWLMFIGVNLTFFPMHFLGLAGMPRRIPDYPDSYA
GWNSVASFGSILSSVASLLFFYIVYLTLTEGHLEESNPWVKD
RGIAFPTIESKSG

Nucleotide Sequence

>AB776657.1 *Sargassum muticum* mitochondrial cox1 gene for cytochrome oxidase subunit 1, partial cds
GGGGATTCGGCAATTGGTTTGTTCCTTTAATGATAGGTG
CTCCAGATATGGCGTTCCCTCGTATGAATAATATAAGCTT
TTGGTTGTTGCCGCCATCTTTAATGCTTCTTTTAGCGTCCT
CATTAGTGGAAGCTGGAGCTGGTACGGGTTGGACCGTTT
ATCCACCGTTAAGTGGTATCCAGGCACATTCAGGCCCATC
AGTCGATTTGGCTATTTTTAGTTTACACTTATCAGGTGCC
GCTTCTATTTTAGGTGCCATTAATTTTATAACAACCATATT
TAATATGCGTGCTCCGGGTATGGGTATGCACCGTTTACC
CTTGTTTGTTTGGTCTGTGTTAATAACAGCATTTTTACTTT
TATTATCCCTTCCGGTTTTGGCCGGAGGTATTACTATGCT
TTTAACGGATCGTAATTTCAATACCACATTTTTTGATCCG
GCGGGGGGTGGTGATCCCGTGCTTTATCAGCATTTGTTT

TGGTTTTTTGGACATCCCGAGGTGTATATTTTAATATTGC
CGGGGTTTGGTATAGTTAGTCATATTTTGGCTACTTTTTC
AAGAAAGCCCGTTTTTGGTTATTTAGGTATGGTTTACGCG
ATGTTGTCTATTGGTATTTTGGGTTTTATCGTTTGGGCTC
ATCACATGTTTACAGTTGGCTTGGATATTGATACGAGAG
CTTATTTTACTGCGGCTACGATGATTATTGCTGTTCCTAC
GGGTATTAAGATTTTTAGCTGGATAGCCACCATGTGGGG
AGGTTCGATACGCCTTAAAACACCGATGCTATTTTCTATA
GGTTTCCTTTTTTTGTTTACTATCGGGGGGTTGACAGGTG
TTGTTTTGGCTAATTCGGGTGTAGATATTGCTCTTCACGA
TACTTATTATGTGGTTGCACATTTCCATTATGTTTTAAGTA
TGGGAGCTGCTTTTACTATGTTTGGTGCTTTTTATTTTTGG
GTTGGGAAAATGACTGGTTTGGGTTACCCGGAAGTTTTA
GGACAAATTCATTTTTGGCTTATGTTTATTGGGGTAAATT
TAACATTCTTTCCTATGCATTTTTTGGGATTAGCTGGCAT
GCCTCGTCGTATTCCAGATTATCCAGACTCGTATGCCGGT
TGGAATAGTGTAGCCTCTTTTGGGTCCATACTCTCTTCAG
TGGCTTCATTGCTTTTCTTTTATATCGTCTATTTGACATTA
ACCGAAGGGCATCTTGAGGAATCTAATCCTTGGGTTAAA
GATAGAGGTATTGCTTTTCCGACGATTGAATCAAAAAGT
GGGTAA

2. Primary Analysis.

A. protscale:

User-provided sequence:

```
        10          20          30          40          50          60
LANSGVDIAL  HDTYYVVAHF  HYVLSMGAAF  TMFGAFYFWV  GKMTGLGYPE  VLGQIHFWLM

        70          80          90         100         110         120
FIGVNLTFFP  MHFLGLAGMP  RRIPDYPDSY  AGWNSVASFG  SILSSVASLL  FFYIVYLTLT

       130         140
EGHLEESNPW  VKDRGIAFPT IESKSG
```

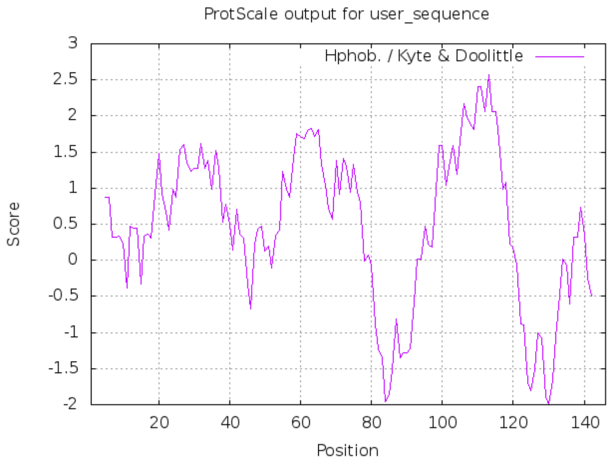
SEQUENCE LENGTH: 146

Using the scale Hphob. / Kyte & Doolittle, the individual values for the 20 amino acids are:

```
Ala:  1.800 Arg: -4.500 Asn: -3.500 Asp: -3.500 Cys:  2.500 Gln: -3.500
Glu: -3.500 Gly: -0.400 His: -3.200 Ile:  4.500 Leu:  3.800 Lys: -3.900
Met:  1.900 Phe:  2.800 Pro: -1.600 Ser: -0.800 Thr: -0.700 Trp: -0.900
Tyr: -1.300 Val:  4.200: -3.500: -3.500: -0.490
```

Weights for window positions 1... 9, using linear weight variation model:

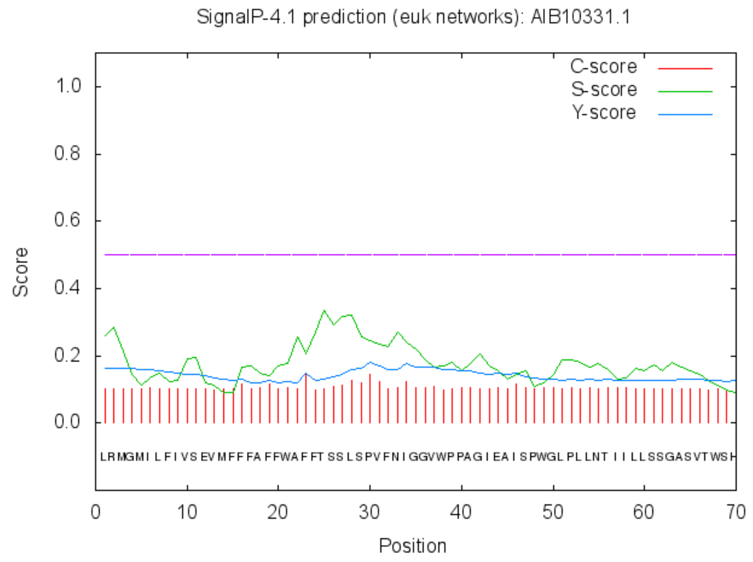
```
  1    2    3    4    5    6    7    8    9
 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00
 Edge           center           edge
```



The above results show the primary analysis of cytochrome oxidase.

B. Signal P

>AIB10331.1 cytochrome oxidase subunit 3, partial _mitochondrion_ _*Sargassum muticum*_



```
# Measure Position Value    Cutoff   signal peptide?
  Max. C    23       0.146
  Max. Y    30       0.181
  Max. S    25       0.335
  Mean S      1-29    0.192
       D     1-29    0.186   0.500   NO
Name=AIB10331.1                                     SP='NO'    D=0.186    D-cutoff=0.500
Networks=SignalP-TM
```

The above result shows the signal regions in cytochrome oxidase.

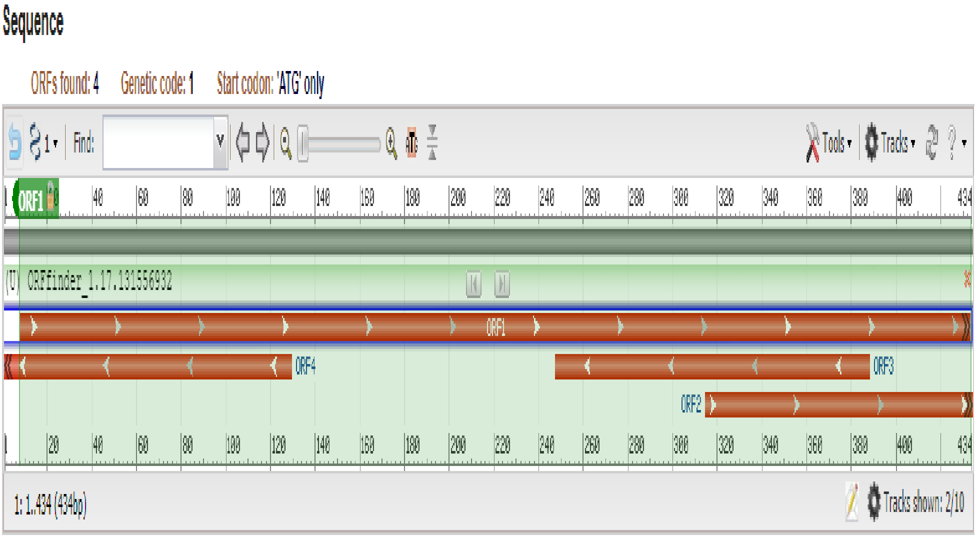
C.ORF finder

ORF Finder results

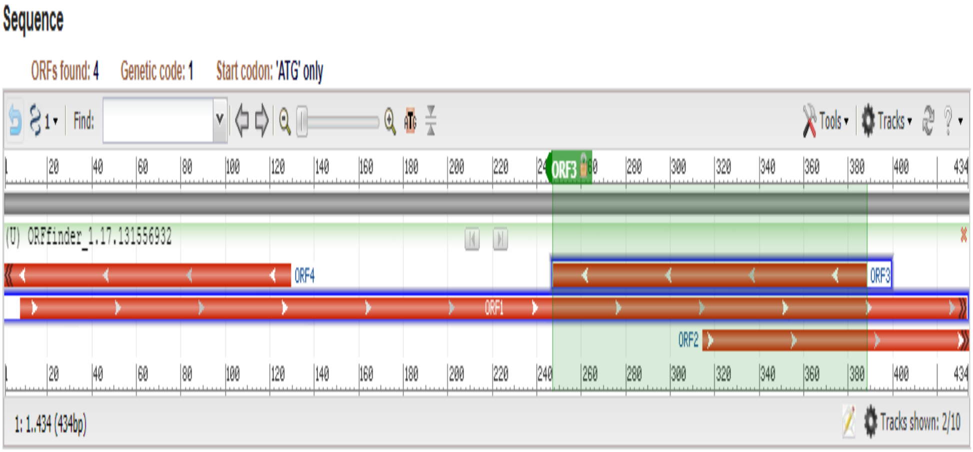
Results for 589 residue sequence "sample sequence" starting "gcggcggcgg"

```
>ORF number 1 in reading frame 1 on the direct strand extends from base 1 to base 93.
Gcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcggcg
Gcggcggcggcggcggcggcggcggcgtaa

>Translation of ORF number 1 in reading frame 1 on the direct strand.
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA*

>ORF number 2 in reading frame 1 on the direct strand extends from base 94 to base 588.
KSargassummutcumvuchrGNTDCcytchrmxdassubuntcxgnartacdsmtchnd
RaCTTGCGCATGGGGATGATTCTTTTCATTGTTTCGGAGGTTATGTTTTTTTTTGCTTTT
TTTTGGGCTTTTTTCACATCCTCTTTGTCCCCTGTTTTTAATATCGGGGGGGTTTGGCCT
CCGGCTGGCATAGAGGCTATTAGTCCATGGGGATTACCTCTTTTAAATACTATTATTTTA
CTTTCTTCCGGCGCTAGTGTCACATGGTCTCATCATGCTATCGTCGGAGGTTTTAAAAAG
GAGGCTCTATTAAGTTTGCTCATTACAATAATATTTGCAGTTATTTTTAMTGGATTGCAG
GGGTTCGAGTATATTAATGCACCTTTCGCTATGTCCGACAGTGTTTATGGTTCTGTTTTT
TTTATGGCTACAGGTTTTCATGGTTTCCATGTTATTATTGGTACCATCTTTTTATCTGTT
TGTACTTTTCGGTTA
```

>Translation of ORF number 2 in reading frame 1 on the direct strand.
XXXXSXXXXXXXXXXXXXXXXLRMGMILFIVSEVMFFFAFFWAFFTSSLSPVFNIGGVWP
PAGIEAISPWGLPLLNTIILLSSGASVTWSHHAIVGGFKKEALLSLLITIIFAVIFXGLQ
GFEYINAPFAMSDSVYGSVFFMATGFHGFHVIIGTIFLSVCTFRL

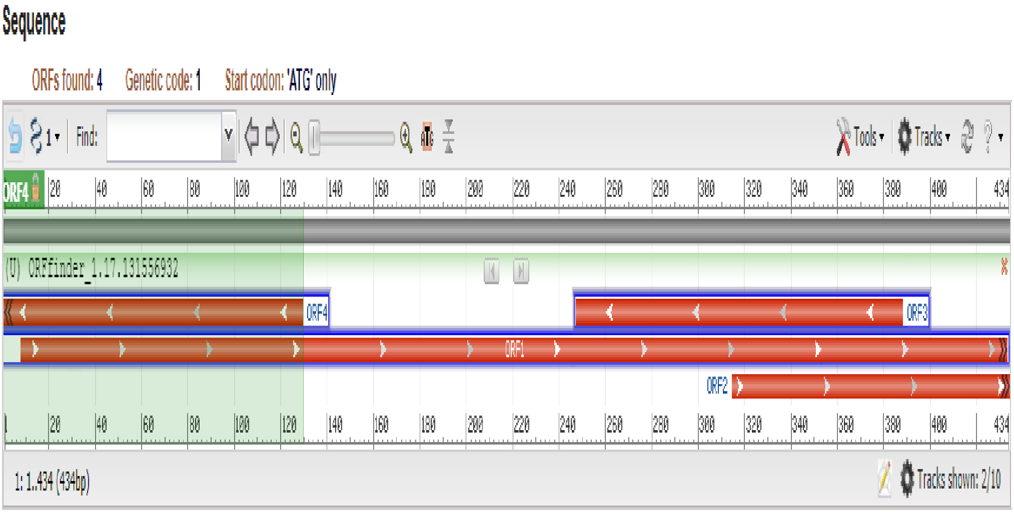


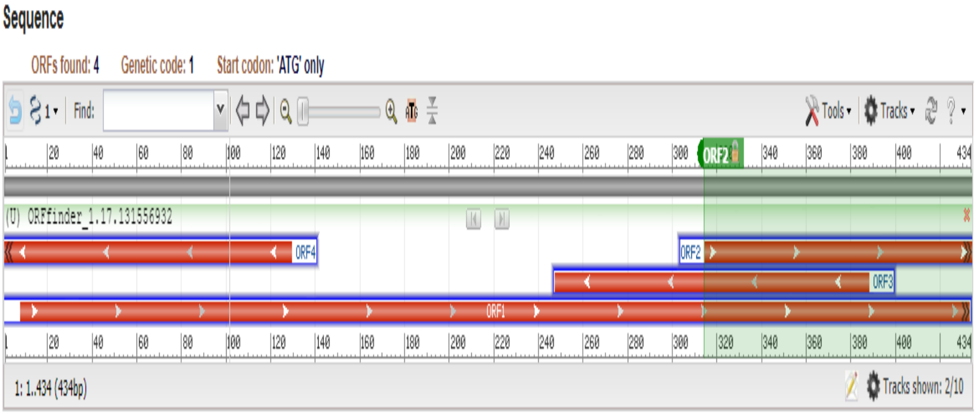
ORF 1:



ORF 3:

ORF 4:





Sequence

ORFs found: 4 Genetic code: 1 Start codon: 'ATG' only

(U) ORFfinder_1.17.131556932

1: 1..434 (434bp) Tracks shown: 2/10

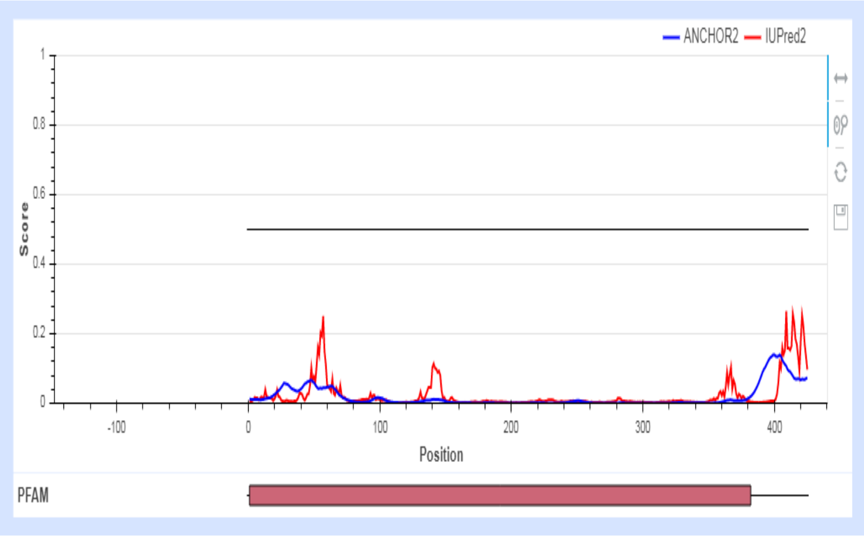
ORF 2:

The above result shows the ORF regions of cytochrome oxidase.

3. ANCHOR TOOL:

Prediction of unstructured Proteins

Prediction of Intrinsically Unstructured Proteins

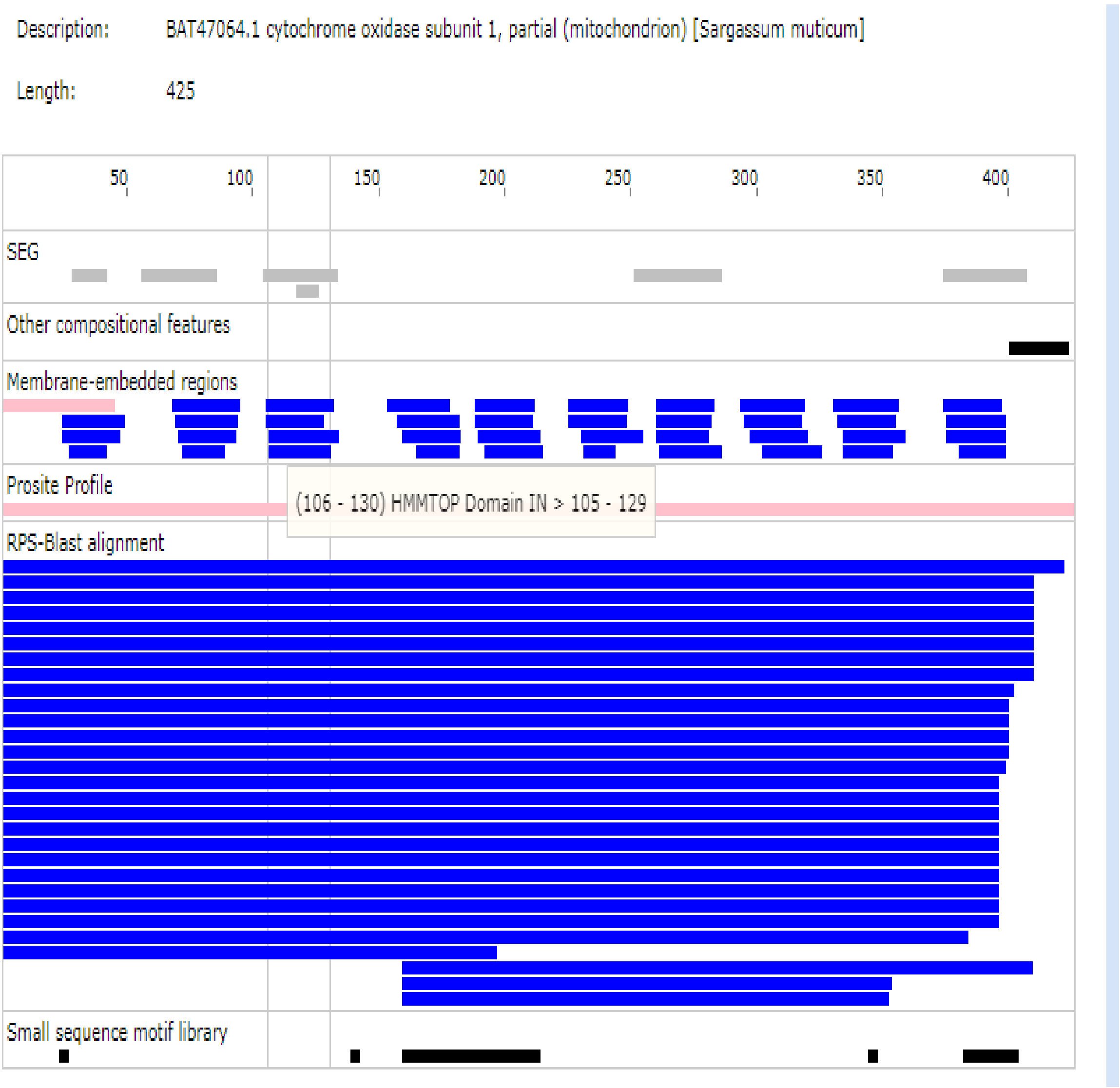


```
# Balint Meszaros, Gabor Erdos, Zsuzsanna Dosztanyi
# Nucleic Acids Research 2018, Submitted
# POS                                   AMINO ACID      IUPRED    SCORE
                                        ANCHOR SCORE
1                                       G   0.0046   0.0123
2                                       F   0.0065   0.0113
3                                       G   0.0070   0.0113
4                                       N   0.0108   0.0110
5                                       W   0.0168   0.0113
6                                       F   0.0148   0.0111
7                                       V   0.0151   0.0107
8                                       P   0.0094   0.0106
9                                       L   0.0136   0.0100
10                                      M   0.0181   0.0103
11                                      I   0.0160   0.0106
12                                      G   0.0171   0.0119
13                                      A   0.0398   0.0132
```

14	P	0.0191	0.0138
15	D	0.0171	0.0147

The above result shows the unstructured unit of cytochrome oxidase.

4. ANNIE tool – Prediction of functional Unit


Description: BAT47064.1 cytochrome oxidase subunit 1, partial (mitochondrion) [Sargassum muticum]

Length: 425

The above result shows the functional units of cytochrome oxidase.

CONCLUSION:

The seaweeds used in human food, cosmetics, fertilizers and extraction of industrial gums. Marine algae may also be used as energy collectors and potentially useful substances may be extracted by fermentation. The cytochrome oxidase is the terminal complex of the electron transfer chain. The protein sequence of cytochrome oxidase was retrieved from NCBI database. Structural and Sequencial analysis of cytochrome oxidase was carried out insilco bioinformatics tools. A comprehensive study of the protein may be further used in research.

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