

The genetic relatedness between red abalone; blacklip and greenlip abalone; medaka and zebrafish

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Summary

- Filtered and analyzed water samples.
- Created Nanodrop Graph.
- Gene expression (COX-1) via PCR and Agarose Gel Electrophoresis.
- Created phylogenetic trees of red, blacklip, and greenlip abalone- medaka, red abalone, zebrafish comparatively.
- Looked at their similarity through their evolutionary process.

Abstract

- Red, greenlip and blacklip abalone are marine organisms and we are interested to study how evolutionarily they are connected to each other. Medaka lives in brackish water, zebra fish in fresh water and red abalone in seawater, that is why, we are interested to see whether red abalone is more evolutionarily connected with zebrafish or to medaka.

Introduction

Hypothesis 1: The Red Abalone (COX-1) sequence will be less similar to that of Greenlip Abalone, and more similar to Blacklip Abalone.

Red Abalone (*Haliotis rufescens*)

- Large, thick, dome-shaped shell which has usually a dull brick color externally. [1]

Greenlip Abalone (*Haliotis laevigata*)

- Got its name for its distinct green ring at the bottom and maximum shell length 24 cm, mostly 14-17 cm [2].

Blacklip Abalone (*Haliotis rubra*)

- The Blacklip Abalone has a dark band along the edge of its shell and lives in the coasts of Australia [3],[4].

Hypothesis 2: The Red Abalone is more similar to the Medaka than the Zebrafish.

Salinity[11]

- Freshwater-->0.5 ppt (Zebrafish)[12]
- Brackish water-->.5ppt to 2ppt (Medaka)[10]
- Seawater--> 33 ppt to 38 ppt. (Red abalone)[13]

COX-1 Gene (Cytochrome C Oxidase Subunit 1)

- Three subunits of COX-1 Gene are ciphered in mtDNA [5].
- The last enzyme in ETC (Electron Transport Chain) transfers electrons from cytochrome c to molecular oxygen [6].
- Mostly used for DNA barcoding.

Materials and Methodology

Tissue Sampling and eDNA Extraction:

- 1 liter of water from the tank of red abalone (Filtered through .45um MCE#HABG04700)
- Filter Frozen at -80C for storage or processed through Mo Bio Power Water Kit for DNA Extraction.

Water Sampling DNA Extraction

- NCBI Database: Green lip abalone (*Haliotis laevigata*), Black lip abalone (*Haliotis rubra*), medaka, zebrafish
- Tissue DNA for medaka and zebrafish

PCR and Primer

- Ran PCR on extracted DNA Samples. 5ul of DNA Template, using Gotaq 2x Protocol. Universal COX-1 or Ab COX-1 primers.55C Annealing temperature used. Primer
- FWD: TGATCCGGCTTAGTCGGAAGTGC
- RVS: GATGTGTTGAAATTACGGTCCGT

Gel Electrophoresis

- Ran PCR samples on agarose gel.
- 10 ul of PCR rxn used + 2ul loading dye
- 1Kb DNA Ladder used (12ul)
- 1.5% Agarose Gel
- 90V for 55 Minutes
- SYBR Safe Stain and UV Box used for visualization

DNA Sequencing and Analysis

- Comparison among the COX-1 genes and DNA sequences are carried out using the BLAST (The Basic Local Alignment Search Tool), NCBI. Furthermore, the phylogenetic trees were generated by importing tree data from Clustal Omega to iTOL. [15][16]

Results

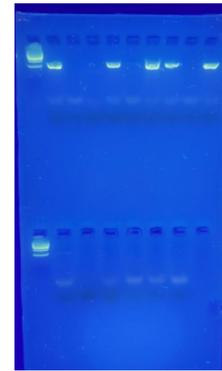


Figure-1: Agarose Gel Electrophoresis Results for COX1 gene of red abalone eDNA, gel electrophoresis was done to see the size of DNA samples and check the PCR results.



Figure-2: The phylogenetic tree (real) of COX1 gene of red, blacklip, and greenlip abalone, which indicates the evolutionary time passed in respective to their common ancestor and taxonomic relationship between the species.



Figure-3: Phylogenetic tree from tissue DNA COX-1 Gene sequences, which indicates the evolutionary time passed in respective to their common ancestor and taxonomic relationship between the species.



- **Figure-4: Phylogenetic tree using DNA sequences from NCBI Database**, indicating the evolutionary time passed in respective to their common ancestor and taxonomic relationship between the species.

Sequence alignments:

Hypothesis 1

- *Haliotis laevigata* → Query cover: 92% Percentage Identities: 84.00%
- *Haliotis rubra* → Query cover: 84%, Percentage identities: 84.44%

Hypothesis 2

- *Oryzias latipes* → Query cover: 86%, Percentage Identities: 72.31%
- *Danio rerio* → Query cover: 90%, Percentage Identities 73.88%

Discussions

- The Hypothesis 1 is correct.
- In figure 3: From the length of the branches of the phylogenetic tree we can imply that red abalone is evolutionarily closely related to the blacklip rather than the greenlip abalone.
- In sequence alignments we can see a slight more connection of blacklip abalone (84%) with red abalone than that of greenlip abalone (83.92%)
- Figure-1 also denotes that blacklip and greenlip abalone are more evolutionarily connected than that of red abalone as red abalone suffers from withering syndrome unlike blacklip and greenlip abalone.
- The Hypothesis 2 is not correct, based on the sequence alignments we have had from NCBI Blast, the Red abalone is more evolutionarily related to the Zebrafish than that of the Medaka.
- The percentage of identities of gene sequences of red abalone with medaka is 72% whereas to that of zebra fish is 74%.
- As a result, salinity is not a factor which affects the evolutionary relationship for these organisms.

Limitations & Future Directions

- In figure-4 and figure-5 a difference in the phylogenetic trees can be observed since in the tissue sequence the whole COX-1 gene was not used.
- No tissue sequence of greenlip and blacklip abalone were collected for this study.
- Future studies on these two hypotheses might help us to understand how greenlip and blacklip abalone had gone through less mutations than that of red abalone.
- Might also provide us with insights why red abalone suffers from the withering syndrome but not blacklip and greenlip abalone.

References

