Molecular phylogeny of labyrinthulomycetes (stramenopiles)
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Labyrinthulids are marine fungal-like heterotrophs, classified in the large eukaryotic super group called stramenopiles. They are of much interest in industry use, because of their high amount production of polyunsaturated fatty acid (PUFAs) and potential source of new bioactive compounds. The systematics of labyrinthulids is well not known, due to their morphological plasticity and complicated life history. Recent taxonomic revision in the class Labyrinthulomycetes has described five new genera based on morphology and profiles of PUFAs and carotenoids, but there are still many remained lineages that taxonomic treatment to be completed. Nuclear small-subunit ribosomal RNA (nSSU rRNA) gene is commonly used for genus- or species-level molecular phylogeny of the class Labyrinthulomycetes. However, we have found some labyrinthulid strains having more than one sequence variation of that gene in their genomes, and direct sequencing of the PCR product is often impossible. At NBRC, we have been developing collections of labyrinthulid strains with being identified into at least genus-level, and trying to develop “easy and fast” genus identification system of labyrinthulids using protein coding gene sequences.

We newly designed primer sets for elongation factor 1-alpha and actin, using gene sequences of some labyrinthulids available in NCBI GenBank database, and we tested them against wide range of labyrinthulid strains available at our NBRC collection. Most PCR products showed a single band on an agarose gel, but sequence chromatograms with double peaks at some codon sites were produced, suggesting at least two copies of these genes in their genomes. However, almost all nucleotide variations occurred at third codon sites with synonymous difference of encoded amino acids.

In phylogenetic trees of each gene, based on amino acid sequences or DNA sequences of combined 1st and 2nd codon positions, each labyrinthulid genus formed a monophyletic clade, and no significant conflict was observed with the nSSU rRNA gene tree. We concluded that these genes are not “perfect” genes but most reliable genes for labyrinthulid systematics so far, because no need of PCR cloning is quite beneficial for us to work on large number of isolates.