

Research Article

The DNA Sequence Encoding Actin (ACT1) of Pandan (*Benstonea* sp.)

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ABSTRACT

One of housekeeping genes is actin gene. This gene is frequently used in gene expression studies as an internal control. The DNA sequence encoding actin from Pandan (*Benstonea* sp.) clone Riau has not been reported, therefore, this study investigated the DNA sequence encoding actin isolated from *Benstonea* sp. clone Riau. Total DNA isolation was performed in fresh leaves, total RNA isolation from stem, total cDNA synthesis, polymerase chain reaction using degenerate actin primer, electrophoresis, cloning, transformation, blue white colony selection, colony PCR, sequencing, data analysis using BioEdit and MEGA6 softwares and BLASTn program. The partial DNA sequence encoding actin from *Benstonea* sp. clone Riau obtained was 1,403 bp. The sequence was grouped as part of actin1 (ACT1) and it consisted of two exons and one intron. The predicted coding and peptide sequences were 616 bp and 205 amino acids, respectively. The predicted coding sequence had 90% similarity to some ACT1 mRNA from some plants species but none of which belongs to *Benstonea* genus or Pandanaceae family. The deduced peptide sequence had similarity to some ACT1 peptide from some plant species of up to 99% and also none of them belongs to *Benstonea* genus or Pandanaceae family. Thus, the partial ACT1 gene obtained in this study was the first sequence reported from *Benstonea* genus. Furthermore, this sequence can be used as a reference to isolate actin genes from other species within *Benstonea* genus for gene expression analysis purposes.

Keywords: ACT1 gene, DNA sequence, *Benstonea* sp., Pandan

Introduction

The *Actin* gene (*Act*) together with tubulin (*TUB*), translation elongation factor (*EF1 α*), cyclophilin (*CAC*) [1, 2], ubiquitin (*UBQ*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), transcription initiation factor (*TIF*), 18S rRNA, and 25S rRNA [1] genes are housekeeping genes which are often used as an internal control for normalization of gene expression. It is because expression of the genes is stable, not influenced by developmental stages, and not induced by certain treatments. In addition, amount of the expressions is abundant in whole tissues at every eukaryotic organism developmental stage [3].

The actin protein is coded by many genes that are called *multigene family*. *Actin* genes in *Oryza sativa* have been identified in chromosomes three

and five [2]. Eight members of *actin* gene family have been detected in *Populus* genus and they are encoding 377 amino acids [4]. Four members of *Melastoma malabathricum actin* genes family, i.e. *MmACT1*, *MmACT2*, *MmACT3*, and *MmACT4* [5] and 10 of *Arabidopsis thaliana* ones [6] have also been identified and characterized. One of *A. thaliana actin* genes, such as *AtACT2*, is most frequent and more accurate to be applied as an internal control in *A. thaliana* gene expression analysis [7].

Benstonea sp. clone Riau is one plant species growing in and around Kajuik Lake [8]. Kajuik Lake is a kind of floodplain lake which is located in Langgam District, Pelalawan Regency, Riau Province, Indonesia. Kajuik Lake is formed by overflow of Kampar River when the water floods to the lower plain around the river in rainy and dry

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seasons. Consequently, many unique floras grow in and around Kajuik Lake. They are adaptive to many kinds of environmental stresses especially flooded stress for a few months. Some other plants that grow in and around Kajuik Lake are trees, such as tuntun angin (*Elaeocarpus floribundus*), rengas (*Gluta renghas*), durik-durik (*Syzygium* sp.), and putat (*Planchonia valida*); rotan (*Calamus* sp.); ferns; and grasses [9, 10, 11, 12].

Four degenerate actin primers have been designed based on *actin* gene conserved regions among the known plant, animal, fungal, and protist, namely PIAc12S and PIAc46S-20 as forward primers and PIAc245N-20 and PIAc284N as reverse primers [6]. The four primers have been applied in *Melastoma malabathricum* [5]. A primer pair of PIAc12S-20 and PIAc245N-20 has also been used to isolate *E. floribundus actin* gene [13]. Furthermore, a primer pair of PIAc46S-20 and PIAc245N-20 has been used to isolate *durik-durik* (*Syzygium* sp.) *actin* gene [14]. However, DNA sequence of *actin* gene on *Benstonea* sp. clone Riau and also on other species in Pandanaceae family has never been reported. Therefore, this study aims to isolate the DNA sequence encoding actin from *Benstonea* sp. clone Riau.

Material and Methods

Materials

Benstonea sp. plant was collected from Kajuik Lake which is located in Langgam District, Pelalawan Regency, Riau Province, Indonesia at coordinates of 0 16 27.2° N, 101 42 30.0° E. This plant was identified by Roslim (2017). A degenerate actin primer pair designed by McDowell *et al.* [6] was used to amplify the actin gene of *Benstonea* sp. clone Riau using the total DNA as template. The primer pair as follows: 46S_F 5'-ATG GTN GGN ATG GGN CAR AA-3' and 245N_R 5'-GTD ATN ACY TGN CCR TCN GG-3'.

Total DNA isolation

The fresh leave total DNA isolation was conducted using DNeasy® plant mini kit (Qiagen, Canada). The total DNA was then checked on 1% agarose gel in 1× TBE buffer according to Roslim *et al.* [15].

Amplification using polymerase chain reaction (PCR) technique

The PCR technique to amplify an actin gene

on *Benstonea* sp. clone Riau was performed in 50 µL PCR reaction with the following components: 1X PCR buffer for KOD FX Neo (Toyobo, Japan), 0.4 mM dNTPs (Toyobo, Japan), 0.3 µM primer forward, 0.3 µM primer reverse, 1 U KOD FX Neo (Toyobo, Japan), and 1 µL the total DNA as template, and distilled water. The PCR process was done with the following conditions: 2 minutes at 94°C for 1 cycle followed by 15 seconds at 94°C, 30 seconds at 47.7°C, and 1 minute at 68°C for 35 cycles. The PCR process was ended with 1 cycle of post-PCR for 10 minutes at 68°C. The PCR products were then checked by electrophoresis.

Electrophoresis

Electrophoresis was performed on 1.0% agarose gel in 1× TBE buffer, at 75 volts for 30 hours using submarine electrophoresis system (Mupid -exU, Japan). The bands were then stained by using 5 µg/mL ethidium bromide solution and visualized on the UV lamp transilluminator (WiseUv WUV-M20, Daihan Scientific). A digital camera (Olympus SP-500 UZ) was used to record the bands.

PCR products cloning and sequencing

The PCR products were purified with Zymo-clean™ Gel DNA Recovery Kit (Zymo Research, China), cloned into pTA2 Vector with Toyobo TArget Clone Plus (Toyobo, Japan) then transformed into *E. coli* Zymo 5a with Mix and Go Competent Cells™ (Zymo Research, China). Plasmid isolations were then performed from *E. coli* Zymo 5a recombinant cells with ZR Plasmid Miniprep (Zymo Research, China). The plasmids were amplified with primer pair of T3 and T7 promoters using KOD FX Neo (Toyobo, Japan) then the PCR products were purified with Zymo-clean™ Gel DNA Recovery Kit (Zymo Research, China). After that, the purified PCR products were sequenced at 1st Base Malaysia with bi-directional sequencing [15].

Data analysis

The complete sequences were obtained by aligning the forward and reverse sequences using BioEdit version 7.0.0 software [16]. Analysis of the sequences using BLASTn program [17] was performed to determine the similarity between the sequences and the sequences were deposited in the GenBank database. The coding DNA region was

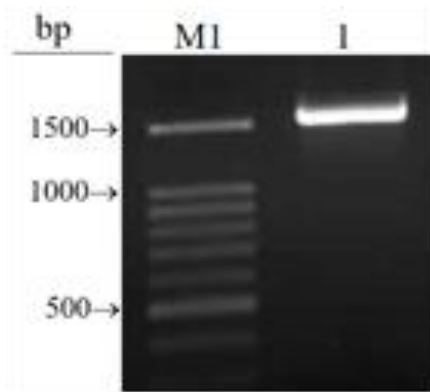


Figure 1. Profile of actin fragment of Pandan (*Benstonea* sp) clone Riau migrated on 1.0% agarose gel. M1 = 1 kb DNA ladder, 1 = the actin fragment.

Table 1. The BLASTn analysis on the DNA sequence encoding actin (*ACT1*) of *Benstonea* sp. clone Riau.

Description	Total score	Ident (%)
<i>Hippeastrum hybrid (ACT1)</i>	747	90
<i>Dendrobium catenatum (ACT1)</i>	691	89
<i>Phalaenopsis hybrid (ACT4)</i>	722	89
<i>Narcissus tazetta (ACT1)</i>	702	89
<i>Mangifera indica (ACT4)</i>	696	89
<i>Camellia sinensis (ACT)</i>	709	89
<i>Morus alba (ACT2)</i>	702	89

predicted through BLASTn result. Analysis of the sequences using MEGA version 6.06 software [18] was then conducted to create a phylogenetic tree by using maximum likelihood statistical method and Kimura 2-parameter model with 1,000 bootstraps.

Results and Discussion

Amplification resulted in the DNA fragment encoding actin with size approximately 1,500 bp (Figure 1). After sequencing, the length of the actin sequence was 1,403 bp. The sequence was already registered in GenBank with the accession number MF327593.1.

Analysis of BLASTn showed that the sequence obtained in this study was the right part of actin gene. The DNA sequence encoding actin of *Benstonea* sp. clone Riau had highest genetic similarity to mRNA sequence encoding actin1 (*ACT1*) of *Hippeastrum hybrid* (max score = 430, total score = 747, query cover value = 44%, E-value = 5e-116, and identity value = 90%) (Table 1). It predicted that the *Benstonea* sp. DNA sequence obtained is part of *ACT1* gene. Moreover,

there were no the DNA sequence data from *Benstonea* genus or Pandanaceae family deposited in GenBank. This result showed that the *ACT1* gene isolated in this study was the first sequence reported from the genus *Benstonea*.

The alignment showed that *Benstonea* sp. *ACT1* sequence obtained in this study consisted of 2 exons and 1 intron. The first exon was in nucleotide 1 – 321 and the second one was in nucleotide 1,105 – 1,403 (Figure 2). The exon total length was 616 bp and intron was 784 bp.

Phylogenetic tree showed that the *ACT1* of *Benstonea* sp. clone Riau formed one group with other actin sequences from many plants species, especially with *ACT1* gene from *Hippeastrum hybrid* and *Narcissus tazetta*. The mRNA encoding ubiquitin from *A. thaliana* were used in the phylogenetic tree analysis as a comparison to sharpen the tree (Figure 3). It is because the ubiquitin is also a member of housekeeping genes [1].

Actin is a microfilament globular protein of eukaryotic cytoskeleton. In general, the actin serves to withstand tension, helps support cells, make the outermost layer of cytoplasm into semi-solid gel, and help the movement of the cell itself and its components [19]. Furthermore, the actin encoding gene is indispensable as an internal control for the normalization of expression data in studies involving measurement of gene expression [1, 2].

Four degenerate actin primers consisting of two forward and two reverse primers have been designed based on the conserved region in plant, animal, and microbe. The four primer positions have also already been known in general structure of *A. thaliana* actin genes [6]. Two of them, namely 46S-20 and 245N-20, have been applied in this study. Both primers are located in exon 2 and 3 and flanking an intron (intron 2) [6].

Amplification of *Benstonea* sp. clone Riau total DNA has produced one DNA fragment which is part of actin gene and comprises two exons and one intron. The result is in accordance with those contained in *A. thaliana* actin genes [6]. The specific actin primers for *Benstonea* sp. clone Riau can then be designed in both exon regions based on the obtained sequence. Such primer designing - namely P_act_F: 5'-TGG TTG GCA TGG GTC AGA A-3' and P_act_R: 5'-GTG ATG ACT TGG CCG TCT GG-3' - is aimed for certain genes expression studies necessity as well as a genomic

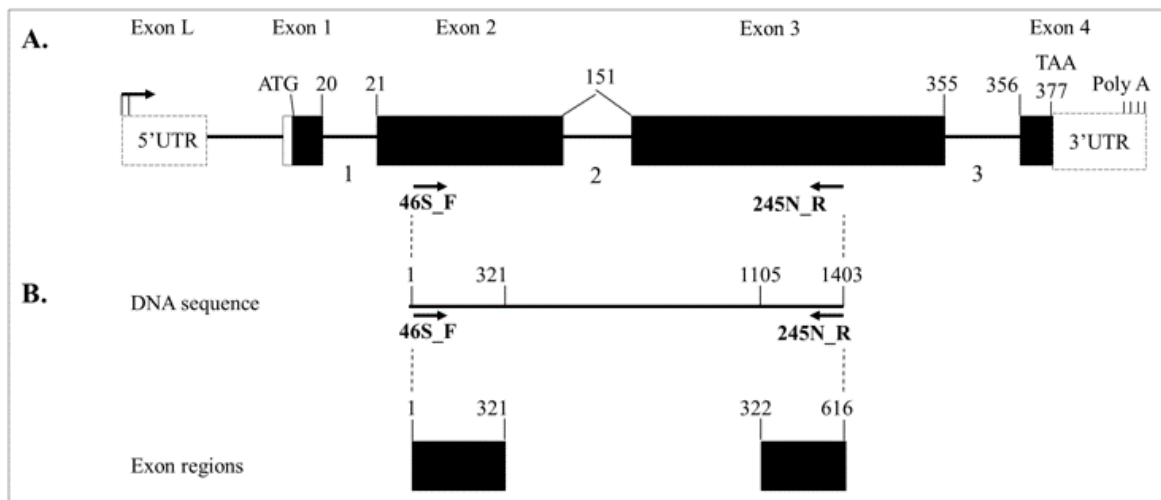


Figure 2. Position of *Benstonea sp.* ACT1 gene referred to general structure of *Arabidopsis thaliana* actin gene. (A) Conserved region physical map of actin gene of *A. thaliana* and an actin primer pair locations. (B) The ACT1 gene of *Benstonea sp.* clone Riau. Boxes indicate exons. Shaded box is the translated region into protein or called exon. 46S_F and 245N_R were an actin primer pair designed of McDowell *et al.* (1996). This primer pair is a universal primer pair for amplification of actin gene in all organisms.

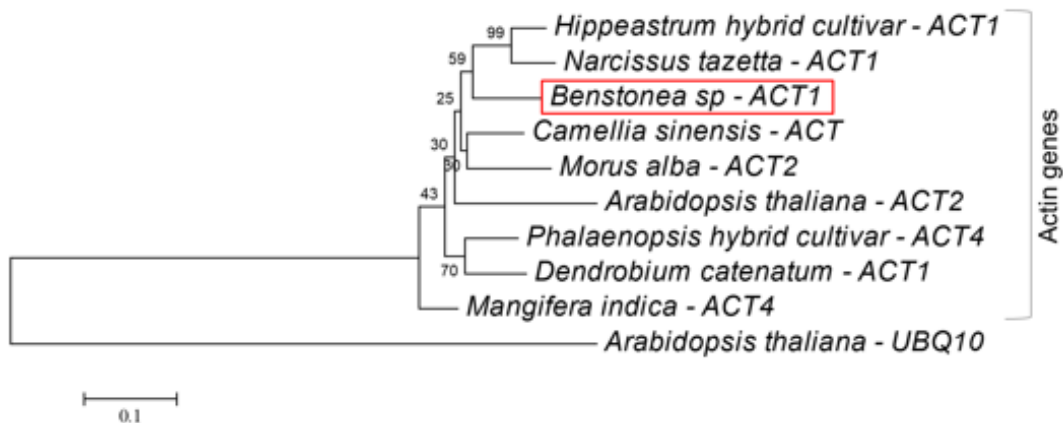


Figure 3. The Phylogenetic tree created based coding sequences of actin. Maximum likelihood statistical method was performed with K2P model and 1,000 bootstraps. ACT = actin gene, UBQ = ubiquitin gene.

DNA contamination verification on a target cDNA [5, 6, 15]. For this purpose, amplification is conducted using DNA and cDNA molecules separately. If the cDNA molecule free from genomic DNA contamination then amplification will result in one amplicon DNA band because cDNA molecule only consists of exon region. On the contrary, if the cDNA molecule is contaminated by genomic DNA then amplification will result in two amplicon bands with discrepancy between the two bands 784 bp (in accordance with intron length on *Benstonea sp.* clone Riau actin gene).

Phylogenetic tree supports the BLASTn analysis result that the ACT1 sequence of *Benstonea*

sp. clone Riau has a higher similarity to actin sequence from another plant species compared to *A. thaliana* ubiquitin sequence. Moreover, the ACT1 sequence obtained in this study is the first sequence reported from *Benstonea* genus and Pandanaceae family. This can be seen from the BLASTn analysis result which does not reveal the actin sequences from *Benstonea* genus or Pandanaceae family (Table 1). Therefore, the ACT1 sequence of *Benstonea sp.* clone Riau can be the reference for actin gene isolation on any other species within the *Benstonea* genus specifically or Pandanaceae family in general.

Conclusion

The partial DNA sequence encoding actin (ACT1) of *Benstonea* sp clone Riau had been isolated, having a size of 1,403 bp. The ACT1 sequence was consisted of two exons and one intron. This gene was the first sequence reported from *Benstonea* genus. Furthermore, this sequence can be used as a reference to isolate actin genes from other species within *Benstonea* genus or Pandanaceae family for gene expression analysis purposes.

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