

Genome sequence analysis of predicted polyprenol reductase gene from mangrove plant *kandelia obovata*

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Abstract. It has been previously reported that dolichols but not polyprenols were predominated in mangrove leaves and roots. Therefore, the occurrence of larger amounts of dolichol in leaves of mangrove plants implies that polyprenol reductase is responsible for the conversion of polyprenol to dolichol may be active in mangrove leaves. Here we report the early assessment of probably polyprenol reductase gene from genome sequence of mangrove plant *Kandelia obovata*. The functional assignment of the gene was based on a homology search of the sequences against the non-redundant (*nr*) peptide database of NCBI using Blastx. The degree of sequence identity between DNA sequence and known polyprenol reductase was confirmed using the Blastx probability *E*-value, total score, and identity. The genome sequence data resulted in three partial sequences, termed c23157 (700 bp), c23901 (960 bp), and c24171 (531 bp). The c23157 gene showed the highest similarity (61%) to predicted polyprenol reductase 2-like from *Gossypium raimondii* with *E*-value $2e^{-100}$. The second gene was c23901 to exhibit high similarity (78%) to the steroid 5- α -reductase Det2 from *J. curcas* with *E*-value $2e^{-140}$. Furthermore, the c24171 gene depicted highest similarity (79%) to the polyprenol reductase 2 isoform X1 from *Jatropha curcas* with *E*-value $7e^{-21}$. The present study suggested that the c23157, c23901, and c24171, genes may encode predicted polyprenol reductase. The c23157, c23901, c24171 are therefore the new type of predicted polyprenol reductase from *K. obovata*.

Keywords: Biosynthetic pathway, dolichol, enzyme, leaf, polyisoprenoid.

1. Introduction

Mangrove forests have long been a rich source of phytochemical compounds producing polyisoprenoid alcohols [1,2] and other secondary metabolites [3,4]. Polyisoprenoid alcohols are classified to polyprenol and dolichol according to stereochemistry. A number of studies have been reported that the major polyisoprenoid in plant leaves were polyprenols rather than dolichols [5,6]. By contrast, in the animal world (especially in livers), dolichols were predominant, and few polyprenols occurred [7,8]



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However, very recently, we are the first group to report that primary polyisoprenoid alcohols of Okinawan and Indonesian mangrove plants were not polyprenols but dolichols [1,2]. Therefore, the occurrence of larger amounts of dolichols even in the leaves of mangrove plants imply that polyprenol reductase is responsible for the polyprenol conversion to dolichol, may be active in mangrove plant leaves. In spite of the ever-present distribution of polyisoprenoid in the plant kingdom, their function plants world remain unclear, especially in mangrove plants. Two polyprenol reductase genes from *Arabidopsis thaliana* have been characterized [9]. However, information on the polyprenol reductase from mangrove species is limited. It, therefore, became necessary to understand the DNA sequence of polyprenol reductase in mangrove plants to obtain more insight into the physiological significance and biosynthetic pathway of these compounds. Here we report the early assessment of probably polyprenol reductase gene from the genome sequence of mangrove plant *Kandelia obovata*.

2. Materials and Methods

2.1. Sample collection

Fresh leaves of *K. obovata* were collected at Okukubi River, Okinawa, Japan. Total RNA was extracted from *K. obovata* leaves using cetyl trimethyl ammonium bromide (CTAB) procedure with minor modification to increase yields as previously described [10]. The total RNA was used for genome sequence.

2.2. Genome sequence analysis of probably polyprenol reductase

Genome sequence of *K. obovata* used shotgun sequencing for sequencing as previously reported [11]. The principle of genome sequencing consists of three steps, short fragment DNA from *K. obovata* were sequenced to a given length of coverage, then continue with genome assembly, and the last step was annotation [11]. Genome sequence of *K. obovata* using a BLAST search led to 3 nucleotide sequences that showed the highest similarity to polyprenol reductase.

2.3. Sequence and data analysis

Three nucleotide fragments named as c23157, c23901, and c24717 then were used for prediction of polyprenol reductase. The functional assignment of three DNA sequences was based on a homology search of the sequences against the non-redundant (*nr*) peptide database of NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) using BLASTX [12]. The degree of sequence identity producing significant alignments between DNA sequences and a known protein database was confirmed by the BLASTX probability score more than 50 bit or an *E*-value $< 10^{-10}$ was considered to have significant similarity [13].

3. Results and Discussion

The three partial sequences of predicted polyprenol reductase from *K. obovata* termed c23157 (700 bp, coding for 228 amino acids), c23901 (960 bp, coding for 306 amino acids), and c24171 (531 bp, coding for 166 amino acids). As shown in Figures 1-3. The amino acid sequence of c23157 showed significant homology to known, predicted polyprenol reductase (Table 1). Among them, the highest homology of *E*-value ($2e^{-100}$), 61%, and 301 bits was depicted with predicted polyprenol reductase 2-like from *Gossypium raimondii*. Similarly, c23901 also showed significant to known, predicted polyprenol reductase as displayed in Table 2. The highest similarity of *E*-value ($2e^{-140}$), 82% identity and 403 bits was shown with Steroid 5- α -reductase DET2 from *Jatropha curcas*.

Furthermore, C24171 has the shortest DNA sequence; producing significant alignment to known, predicted polyprenol reductase from various plants (Table 3). Among them, the highest similarity of *E*-value ($7e^{-21}$), 79% identity, and 93.6 bits score was indicated to polyprenol reductase 2 isoform X1 from *J. curcas*. These results suggested that c23157, c23901, and c24171 encoded probably polyprenol reductase. The c23157, c23901, and c24171 are therefore the new type of predicted polyprenol reductases from *K. obovata*.

It has been reported that a common rule of two sequences are homologous if they are more than 30% matching over their whole lengths, the 30% condition misses many easily identified homologs [13]. In this context, three partial sequences showed more than 61% similarities to known polyprenol reductase in the plant kingdom. These results support previous results that primary polyisoprenoid alcohol in mangroves are dolichol, but not polyprenols where dolichols were found in examined mangrove samples [1-2]. Besides, the composition of polyisoprenoid may be an expression of the distribution of tissues in these mangrove plants [2].

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      10      20      30      40      50      60      70      80
cttctgctagtgtagaagttcataattccgcagagattcttcatgcacttctacgttttggtgtgtgtgtggaccacttt
L L L V * K F I I P Q R F F M H F Y V L G V V W T T F

      90     100     110     120     130     140     150     160
cttgctggtctttaaattggcactatgcatataacatggctccattgggttttgactcatcggtaccctccgctgcta
L L V L N W H Y A Y N M A P L G F D S S D Y P S A A

      170     180     190     200     210     220     230     240
gtcacttgattgaagggttcagttacgttttccctgcacgagcttattttacctgctgcaaagcatctttacaaggtttgg
S H L I E G S V T F S L H E S Y L P A A K H L Y K V W

      250     260     270     280     290     300     310     320
ctagctgtgttttcgcttttattaatggaaatgcatgtctcaaggcgcttttacgagacagtgcatgtcttttaagtacag
L A V F S L L L M E M H V S R R L Y E T V H V F K Y S

      330     340     350     360     370     380     390     400
ctcttcagctcgaatgcacatttttggctatctgacaggccttttttctatacagcagcaccctgtgcatattgtcact
S S A R M H I F G Y L T G L F F Y T A A P L C I C H

      410     420     430     440     450     460     470     480
tttgtgtattggatgctcttaattttgcagctgaccgagcaatgcagttgagtgtagaagtgaaagagaagttgccatct
F C V L D A L N F A A D R A M Q L S V R S E E K L P S

      490     500     510     520     530     540     550     560
attgacttcaattggcaggcatatacattaaacctatcacaaagcttagtggtggtgactgggctggtgctgttattcac
I D F N W Q A Y I K P I T K L R W W Y W A G A A L F T

      570     580     590     600     610     620     630     640
ttgggggtggctgcatcaatacatatgtcatgtgattttgggctctttgcgagaaaacaaggaaaaaactgatgaatatg
W G W L H Q Y I C H V I L G S L R E N K E K T D E Y

      650     660     670     680     690     700
tgattctcatggtgactggtttgaactgtttcctgtccacactacttggtgagatg
V I P H G D W F E L V S C P H Y L A E M

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Figure 1. DNA sequence of *K. obovata* predicted polyprenol (c23157) and its translation to amino acid sequence

Table 1. Distribution of the top 5 Blastx hits on c23157 sequence

Accession	Description	Identity (%)	Total score	E-value
XP_012457241.1	Predicted polyprenol reductase 2-like <i>Gossypium raimondii</i>	61	301	2e-100
XP_016724522.1	Predicted polyprenol reductase 2-like isoform X3 <i>G. hirsutum</i>	62	301	3e-100
XP_016724521.1	Predicted polyprenol reductase 2-like isoform X2 <i>G. hirsutum</i>	62	301	5e-100
XP_016724516.1	Predicted polyprenol reductase 2-like isoform X1 <i>G. hirsutum</i>	62	301	6e-100
XP_002531516.1	Predicted polyprenol reductase 2 isoform X1 <i>Ricinus communis</i>	62	298	2e-99

K. obovata green and yellow leaves have been shown to contain polyprenols C₇₅₋₁₀₀, C₄₅₋₉₅, respectively, while dolichol was C₇₀₋₁₀₀, C₇₅₋₁₀₀, respectively. In contrast, in the roots, no polyprenols detected, with 100% dolichols were C₈₀₋₁₀₅ [1]. This study indicated dolichol is more dominant than polyprenols in green leaves in the ratio of 76.3:23.7 and in the yellow leaves in proportions 42.9:57.1 [1]. In this context, the predominance of dolichol over polyprenol in *K. candel* and the majority of mangrove species suggested that existence of polyprenol reductases in mangrove leaves, which catalyze the conversion of polyprenol to dolichol corresponding to the SRD5A3 protein in animals [14], differ from those of other mangrove plants in reduction activity.

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      10      20      30      40      50      60      70      80
tttttcctttttttttttttatgaacactgaaacaccagttttcctttgcgattgtattccaagtaacatacatctgaa
F F L F F F F M N T E T P V F F C D C I P S N I H L K

      90     100     110     120     130     140     150     160
aacatcagattacagactaaaaaaagaaattgcttcaatacaggaaaggtatcacagctttcctactcttgggataatct
T S D Y R L K K E I A S I Q E R Y H S F P T L G I I

     170     180     190     200     210     220     230     240
tcccaaacttctccagataccactgatgattggcacgtgccctaggaaccaagttggcacacgtgtagagaagaaaccc
F P K L L Q I P L M I G T C P R N Q V G T R V E K K P

     250     260     270     280     290     300     310     320
aaaccgacccagaccacgtcatcagagcccaaccagtcattccacaatctcccaaagtagttgggacagctcacca
K P D P R P R H Q S P T S P F H N L P K V V G T A H Q

     330     340     350     360     370     380     390     400
actcaaaccatcctcctcctcgccaccttgtaccaccacctcctccttcaaggccaatatgaccttatcagcccaaata
L K P S S S R H L V P T T S S L Q G Q Y D L I S P N

     410     420     430     440     450     460     470     480
ttgatccacatgccgatcgcaaaaaccaacaacccaacaacaaacctatacgaaaaccacccatcgccgtcatagtcac
I D P H A D R K N Q Q P N N K P I R K P P I A V I V I

     490     500     510     520     530     540     550     560
cttatagtgactaaccaccttgccctgcaagtaccattcaacagattgtaccaaaagccatgagggcaacgctgaccg
L I V T N P P C L Q V P I Q Q I V P K S H E G N A D R

     570     580     590     600     610     620     630     640
gaaaaccaccgcctgttttgagatcttttgagtggattccggtagagcaggagtgggtagaggcaagtgcgctggaaa
K T T A C F G D L L S G I P V E Q E W V E A S A L E

     650     660     670     680     690     700     710     720
tagtggatgagaaaaggggacatgagaatcagtgctttgggattggtgaagtgtcgacctatggggaagaggagtggt
I V D E K R G H E N Q C F G I G E V S T Y G E K E E C

     730     740     750     760     770     780     790     800
gagccagaggggtggggctttccatgaggaaccaggccagtgggcgcccatgggtggggcccccattcctggacggctgtgct
E P E G G A F H E E P G Q W R R H G G A P S W T A V L

     810     820     830     840     850     860     870     880
ttccgtacgggtgcttgaaggaacctgagagagatgaagggtgggaatgctatgaggtagtgagaaaggaggcgtagtga
S V R C L K E P E R D E G W E C Y E V V R K E G V V

     890     900     910     920     930     940     950     960
aaaatcttctcatcgagacggccattttgatgttgattgaggggggtgatctaacgggtgagtggaacacgtcttgt
K N L L I G D G H F D V D * G G D L T V S G K N V L

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Figure 2. c23901 predicted polyprenol reductase sequence and its amino acid sequence from *K. obovata*

Table 2. Distribution of the top 5 Blastx hits on c23901sequence

Accession	Description	Identity (%)	Total score	E-value
XP_012087922.1	Steroid 5-alpha-reductase DET2 <i>Jatropha curcas</i>	78	403	2e-140
OAY43658.1	Hypothetical protein MANES_08G087200 <i>Manihot esculenta</i>	78	402	3e-140
XP_008370052.1	Predicted steroid 5-alpha-reductase DET2 <i>Malus domestica</i>	76	385	1e-133
XP_002323554.1	3-oxo-5-alpha-steroid 4- dehydrogenases family protein <i>Populus trichocarpa</i>	79	385	6e-100
XP_008355710.1	Predicted steroid 5-alpha-reductase DET2-like <i>M. domestica</i>	62	298	2e-99

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      10      20      30      40      50      60      70      80
gacatttataacctaataatgtaagttgatacaaaaaataaaacaagacttgccacttgccactcgaaagccaaggcttaa
D I Y N L K C K L I Q K I K Q D L P L A T R K P R L K

      90     100     110     120     130     140     150     160
acattataaagaaacctatattgcaagctgcaaaagatgaatacaatttaataatggtgtccggcatagatttcctcggc
H Y K E T Y L Q A A K D E Y N L I N G V R H R F P R

      170     180     190     200     210     220     230     240
ttctttacacgcacagaggcttgttaccatacatcaacttctgtacatcatggtcccagcccctaagagcaagaggtgca
L L Y T H R G L L P Y I N F C T S W S Q P L R A R G A

      250     260     270     280     290     300     310     320
gaactaattaaaaacatagctacttgatctccagcatcataccactgcatttcactcaggcagacatgaaaattaacag
E L I K K H S Y L I S S I I P L H F T Q A D M K I N R

      330     340     350     360     370     380     390     400
gtattcgaacttttgtcatcagttactgctccatcaagctgctgaatcgactaataaacaaggaacacagtcctcgctg
Y S N F C H Q L L L H Q A A E S T N K Q K E T Q S V

      410     420     430     440     450     460     470     480
atttcgtggataatcctcgaacttgtgcaggtaccacctctgtgtttctgctgctgcaaataagagatttgccaccacaa
D F V D N P R T C A G T T S V F L L L Q M R D L P P Q

      490     500     510     520     530
atagaagaagaagccacatgaggatgtcactacctccactggcaactaa
I E E E A T * G C H Y L H W Q L

```

Figure 3. Nucleotide and amino acid sequence of predicted polyprenol reductase (c24171) from *K. obovata*

Table 3. Distribution of the top 5 Blastx hits on c24171 sequence

Accession	Description	Identity (%)	Total score	E-value
XP_012065467.1	Polyprenol reductase 2 isoform X1 <i>J. curcas</i>	79	93.6	7e-21
XP_002531516.1	Predicted polyprenol reductase 2 isoform X1 <i>R. communis</i>	81	92.0	3e-20
XP_009400472.1	Predicted polyprenol reductase 1 isoform X2 <i>Musa acuminata</i> subsp. <i>malaccensis</i>	75	89.4	3e-19
XP_009400470.1	Predicted polyprenol reductase 1 isoform X1 <i>M. acuminata</i> subsp. <i>malaccensis</i>	75	89.0	2e-19
KVH95971.1	3-oxo-5- α -steroid 4-dehydrogenase C-terminal <i>Cynara cardunculus</i> var. <i>scolymus</i>	75	87.8	7e-19

4. Conclusion

The current study suggested that the c23157, c23901, and c24171 genes may encode predicted polyprenol reductase from *K. obovata*. A detailed molecular cloning of the c23157, c23901, and c24171 genes are required further investigation to provide product confirmation of these genes and biosynthetic pathway of polyisoprenoid in mangrove plants.

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