# Molecular Cloning and Bioinformatics Analysis of 2, 4-dienoyl-CoA Reductase1 Gene in Pig

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***Abstract*—2, 4-dienoyl-CoA reductase 1 (DECR1) is an auxiliary enzyme of β-oxidation, and it participates in the metabolism of polyunsaturated fatty enoyl-CoA esters. The full-length cDNA of DECR1 was first cloned from Mashen pig liver, and bioinformatics analysis were then conducted to predicted the physicochemical property, homologous analysis, modification sites and structure of DECR1 protein. Sequence analysis showed that the full-length cDNA of DECR1 was 2352 bp long with an open reading frame (ORF) of 987 bp encoding 328 amino acid residues. Homologous analysis showed that the amino acids of Mashen pig DECR1 shared 99%, 88% , 88% , 87%, 87%, 87%, 87% and 83% identity to Sus scrofa (Predicted), Bos taurus, Homo sapiens, Macaca mulatta, Pan troglodytes, Equus caballus, Canis and Mus musculus, respectively. Bioinformatics analysis showed that DECR1 was a trans-membrane protein, and molecular mass, theoretical point, aliphatic index, estimated half-life, and instability index were 35.43 kDa, 9.37, 85.70, 30 h and 29.06, respectively. Predicted DECR1 have 10 Ser, 6 Thr and 1 Tyr phosphorylation sites. Structure prediction showed that DECR1 consisted of 39.3% α-helix, 11.6% β-extended strand and 49.1% random coil in binary structure, and 2 three and a half α-helices and two 310-helices in three-dimensional structure.***

***Keywords-2, 4-dienoyl CoA reductase 1 (DECR1); cDNA cloning; bioinformatics analysis; pig (Sus scrofa)***

## I. INTRODUCTION

β-oxidation refers to the enzymatic process by which Acyl-CoA is catalytically broken down to yield Acetyl-CoA, the first molecule required for the Krebs cycle. 2, 4-dienoyl-CoA reductase1 (DECR1), originally described by Kunau and Dommes [1], is a mitochondrial protein that belongs to the family of short-chain dehydrogenases/reductases, and serves as auxiliary enzyme of β-oxidation where it participates in the metabolism of polyunsaturated fatty enoyl-CoA esters having double bands in both even- and odd-numbered positions. Specifically, DECR1 uses $NADP^+$ to catalyze the reduction of 2, 4-dienoyl-CoA to yield trans-3-enoyl-CoA, which can then be used as an intermediate in the Krebs cycle [2]. According to the present concept, DECR1 may be the control site of polyunsaturated fatty acids (PUFAs) oxidation. This would be consistent with the importance of the enzyme activity for human survival, because deficiency of DECR1 is lethal [3].

The *DECR*1 has been cloned and characterized in many species including *Homo sapiens* and *Mus musculus, Bos Taurus*, *Equus caballus* and *Canis* [4, 5]. In pig, it has been known that *DECR*1 gene locates on chromosome 4q1.2 between genomic interval 71 cM to 86 cM [6, 7], which coincides with the linoleic QTL associating with the metabolism of fatty acid [8, 9]. Polymorphisms of the pig *DECR*1 have been identified and associated with carcass and meat quality traits [10]. Recent studies revealed that the polymorphism of *DECR*1 was also associated with growth rate in pigs [11, 12]. Pig *DECR*1 gene therefore was thought to be one of the candidate genes for carcass, meat quality and growth traits.

In view of these indispensable functions, little is known about the molecular properties of DECR1. In this work we report the molecular cloning and characterization of the gene for the pig 2, 4-dienoyl CoA reductase 1.

## II. MATERIALS AND METHODS

### A. Cloning of pig DECR1 cDNA

Total RNA of the liver from Mashen pig was extracted. Target fragments were cloned by Reverse Transcription (RT) PCR and rapid amplification of cDNA ends (5′ and 3′ RACE) for sequencing. The resulted nucleotide sequences were assembled to full-length cDNA by SeqMan program of DNAStar software.

### B. Sequence analysis of DECR1

Sequence alignments, ORF translation of the predicted protein were performed online at the website of http:// genes. edu/GENSCAN.html . Clustal *X* and BioEdit v7.0.9.0 software were used for multiple alignment analysis of *DECR*1 amino acid sequences. The molecular mass, isoelectric point, instability index and aliphatic index were predicted online (http://www.expasy.org/tools/protparam.html). The prediction of DECR1 physicochemical property was accomplished by Antheprot software. The prediction of phosphorylation sites were carried out on website of http:// www.cbs.dtu.dk/services/NetPhos/. Predicted binary structure was generated online （http://bioinf.cs.ucl.ac.uk / psipred / psiform.html）. Homology-based structural modeling was performed by Swiss-Model, and Swiss-Pdb Viewer software was used to evaluate the predicted model.

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## III. RESULT

### A. Molecular cloning of the DECR1 full-length cDNA

The full-length cDNA of *DECR*1 (GenBank number: HM004547) was 2352 bp in length, containing a 987 bp ORF, which encoded a protein of 328 amino acids with 53 bp 5′-UTR and 1312 bp 3′-UTR.

### B. Characterization of DECR1 protein

The predicted DECR1 protein had a calculated molecular mass of 35.43 kDa, a theoretical pI at 9.37, aliphatic index of 85.70, estimated half-life of 30 h and instability index of 29.06 (<40). It is indicated that DECR1 DECR1 was a trans-membrane protein and had high hydrophobicity and stability. Alignment analysis showed that the amino acids of Mashen pig DECR1 shared 99%, 88% , 88% , 87%, 87%, 87%, 87% and 83% identity to *Sus scrofa* (Predicted), *Bos taurus*, *Homo sapiens*, *Macaca mulatta*, *Pan troglodytes*, *Equus caballus*, *Canis* and *Mus musculus*, respectively (Fig. 1).

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Mashen pig    ---------- ---------- ---------- ---------- ---------- ----------  [ 60]
Mus musculus  ---------- ---------- ---------- ---------- ---------- ----------  [ 60]
Canis         ---------- ---------- ---------- ---------- ---------- ----------  [ 60]
Homo sapiens  ---------- ---------- ---------- ---------- ---------- ----------  [ 60]
Pan troglodytes ---------- ---------- ---------- ---------- ---------- ----------  [ 60]
Equus caballus---------- ---------- ---------- ---------- ---------- ----------  [ 60]
Macaca mulatta---------- ---------- ---------- ---------- ---------- ----------  [ 60]
Bos taurus    ---------- ---------- ---------- ---------- ---------- ----------  [ 60]
Sus scrofa    MAHKQQKFIA YSSGGWKSMI RVPPADSPFL VCGLPTSHYK VNREQENVWE RSSWAVPLGS  [ 60]

Mashen pig    ---------- ---------- ---------- ---------- ---------- ---MALLGRV  [120]
Mus musculus  ---------- ---------- ---------- ---------- ---------- ----.....A  [120]
Canis         ----------MSEVSQGCL QPIVEGIPSG FGNPWFL--- ---------- ---KKIP..K  [120]
Homo sapiens  ---------- ---------- ---------- ---------- ---------- ----.K.PA..  [120]
Pan troglodytes ---------- ---------- ---------- ---------- ---------- ----.K.PA..  [120]
Equus caballus---------- ---------- ---------- ---------- ---------- ----...P...  [120]
Macaca mulatta---------- ---------- ---------- ---------- ---------- ----.T.QA..  [120]
Bos taurus    ---------- ---------- ---------- ---------- ---------- ----.V.NQL  [120]
Sus scrofa    DPCRRAVLLR QAVLAARGQT AAKASLLPAQ FVEPGYFRPA PCAPARSCAE RQN.......  [120]

Mashen pig    FAPG------ ---------- HGPRRFFTYG TKILYQSTEA FPSKSFPPFQ KVMLPPNTFQ  [180]
Mus musculus  .FA.VSRLPC D--------- P..Q...SF. ..T....KD. PQ..F.Q.VL .P....DA..  [180]
Canis         RSAFSAVLRC SLSEVMVRLG IQNAW..S.. .Q....NN.. .Q..F...L. .A.....S..  [180]
Homo sapiens  .FTLGSRLPC ---------G LA.....S.. ......N... LQ..F.S.L. .A.....S..  [180]
Pan troglodytes .FTLGSRLPC ---------G LA.....S.. ......N... LQ..F.S.L. .A.....S..  [180]
Equus caballus.FTR------ ---------V FD.....S.. ...-..NI.. .Q..F...L. .A.....S..  [180]
Macaca mulatta.FALGSRLPC ---------G LA.....S.. ......N... LQ..F...L. .A.....S..  [180]
Bos taurus    LFSWGR---- ---------- -.S....S.. ..T...NN.. LQ..F....P .....A.S..  [180]
Sus scrofa    ....------ ---------- .......... .......... .......... ..........  [180]

Mashen pig    GKLAFITGGG TGIGKRMTIH LSSLGAQCVI ASRNIDILKA TAEEISSQTG NKVHAIQCDV  [240]
Mus musculus  ..V....... ..L..A...F ..T....... ......V... .......K.. ......R...  [240]
Canis         ..V....... ..L..G..AL .......... ......V..D ...Q...... .........I  [240]
Homo sapiens  ..V....... ..L..G...L .......... ...KM.V... ...Q...... ..........  [240]
Pan troglodytes ..V....... ..L..G...L .......... ...KM.V... ...Q...... ..........  [240]
Equus caballus..V....... ..L..G...L ......R... ...KM.V..V ..DQ...... ..........  [240]
Macaca mulatta..V....... ..L..G..AL .......... ...KM.V... ...Q...... ..........  [240]
Bos taurus    ..V....... ..L..G...C .......... ...K..V..V ...Q...... ..........  [240]
Sus scrofa    ..V....... .......... .......... .......... .......... ..........  [240]

Mashen pig    RDPNMVQNTV SELIKVIGHP DIVINNAAGN FISPSERLSP NAWKTITDIV LNGTAFVILE  [300]
Mus musculus  ...D..M... L.....A... .V........ ........T. .G........ .....Y....  [300]
Canis         .N.E...K.. ......A... A......... ....T...A. .......... .....Y....  [300]
Homo sapiens  ...D...... ......A... N......... ....T..... .......... ..........  [300]
Pan troglodytes ...D...... ......A... N......... ....T..... .......... ..........  [300]
Equus caballus...E...... ......IA.. .......... ....T..... .......... ..........  [300]
Macaca mulatta...D...... ......A... N......... ....T..... .......... ..........  [300]
Bos taurus    ...D...A.. ..T....... .......... .......... .......... .........A  [300]
Sus scrofa    .......... .......... .......... ....T..... .......... ..........  [300]

Mashen pig    IGKQLIKAQK GAAFLAITTI YAETGSGFVV PSASAKAGVE AMSKSLAAEW SKYGMRFNVI  [360]
Mus musculus  .......... .......... ...S.....M ..S...S... ..N..... GR......I.  [360]
Canis         .......... .......... F..S...... .......... ..N..... G.........  [360]
Homo sapiens  .......... .......... .......... .......... ...A..... G.........  [360]
Pan troglodytes .......... .....S.... .......... .......... .......... G.........  [360]
Equus caballus.......... .......... .......... .......... ..N..... G.........  [360]
Macaca mulatta.......... .......... .......... .......... .......... G.........  [360]
Bos taurus    ...E...... ...S...... .......... .......... .LN..... G.........  [360]
Sus scrofa    .......... .......... .......... .......... .......... ..........  [360]

Mashen pig    QPGPIKTKGA FSRLDPTGAF EKEMIDRIPC GRLGTVEELA NLATFLCSDY ASWINGATIR  [420]
Mus musculus  .......... ........R. .......... .....M.... .......... .......V..  [420]
Canis         .......... ........T. ..D..... .......... ...A...... .......V..  [420]
Homo sapiens  .......... ........T. .....G.... .......... ...A...... .......V.K  [420]
Pan troglodytes .......... ........T. .....G.... .......... ...A...... .......V.K  [420]
Equus caballus.......... .......... ...D..N... .......... ...A...... .......V..  [420]
Macaca mulatta.......... ........T. .......... .....I.... ...A...... .......VVK  [420]
Bos taurus    .......... .......... .......... .......... ...A...... .......V..  [420]
Sus scrofa    .......... .......... .......... .......... .......... ..........  [420]

Mashen pig    FDGGEQVLLS GEFNMLRKVT KEQWDTIEGL IRKIKGS [457]
Mus musculus  .....E.F.. ....S.K... ..E..I.... ....... [457]
Canis         .....E.FI. ....S..... ..E..I.... ....... [457]
Homo sapiens  .....E..I. ....D..... ........E. ....... [457]
Pan troglodytes .....E..I. ....D..... ........E. .....V. [457]
Equus caballus.....E..I. ....S.S... .......... ....... [457]
Macaca mulatta.....E..I. ....D..... .......... ....... [457]
Bos taurus    .....E..I. ....S..... .D...I.... ....... [457]
Sus scrofa    .......... .......... .......... ....... [457]
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Mashen pig: HM004547; Mus musculus:NM_026172; Canis:XM_535127; Homo sapiens: NM_001359; Pan troglodytes: XM_001138969; Equus caballus: XM_001488668; Macaca mulatta: XM_001085155; Bos taurus: NM_001075423; Sus scrofa: XM_001924384 (PREDICTED)

Figure 1. Alignment of amino acid sequences of DECR1 proteins

As showm in Fig. 2, the predicted DECR1 physicochemical property included combined antigenicity, hydrophobicity profile, antigenicity profile, hydrophilicity profile, transmembranous regions profile and solvent accwssibility profile. Two potential antigenicity regions of 65-75 site and 220-230 site were identified by Antheprot.

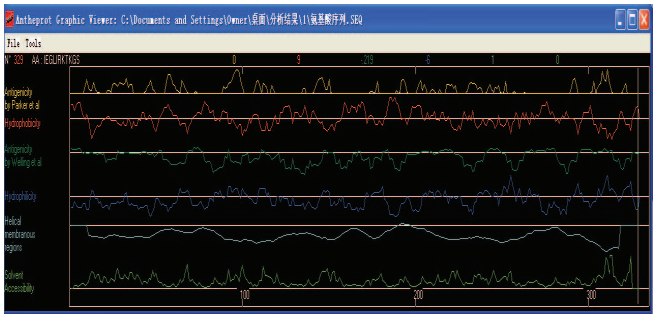
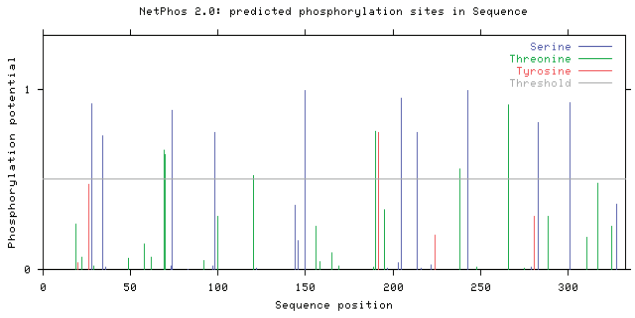



Figure 2. Prediction results of physicochemical property of DECR1

The phosphorylation sites were predicted at Ser, Thr and Tyr site in amino acids encoded *DECR*1 as shown in Fig. 3. Secondary structure analysis showed that DECR1 consisted of 39.3% α-helix, 11.6% β-extended strand and 49.1% random coil (Fig. 4). Molecular homologous modeling of DECR1 for Shanxi Mashen was performed by Swiss Model Server. The result indicated that three-dimensional structure consisted of 2 three and a half α-helices and two 310-helices (Fig. 5). Subsequently, by using Swiss-Pdb Viewer, ramachandran plot evaluation showed that the predicted DECR1 model was rational (Fig. 6).




Serine predictions

| Name | Pos | Context | Score | Pred |
|---|---|---|---|---|
| | | v | | |
| Sequence | 28 | ILYQSTEAF | 0.920 | *S* |
| Sequence | 34 | EAFPSKSFP | 0.745 | *S* |
| Sequence | 74 | THLSSLGAQ | 0.883 | *S* |
| Sequence | 98 | EEISSQTGN | 0.763 | *S* |
| Sequence | 150 | SERLSPNAW | 0.995 | *S* |
| Sequence | 205 | VPSASAKAG | 0.953 | *S* |
| Sequence | 214 | VEAMSKSLA | 0.759 | *S* |
| Sequence | 243 | KGAFSRLDP | 0.996 | *S* |
| Sequence | 283 | SDYASWING | 0.816 | *S* |
| Sequence | 301 | QVLLSGEFN | 0.929 | *S* |
| | | ^ | | |

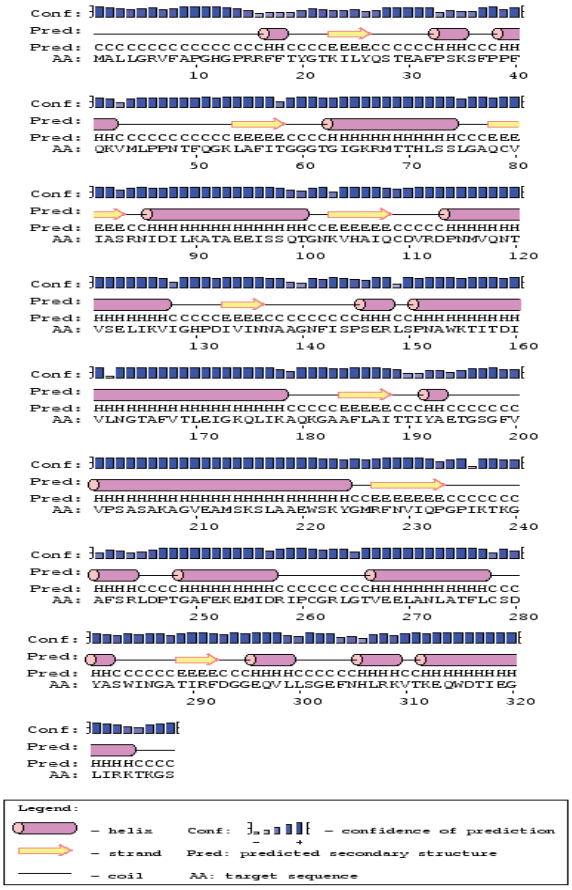
Threonine predictions

| Name | Pos | Context | Score | Pred |
|---|---|---|---|---|
| | | v | | |
| Sequence | 69 | GKRMTTHLS | 0.661 | *T* |
| Sequence | 70 | KRMTTHLSS | 0.637 | *T* |
| Sequence | 120 | MVQNTVSEL | 0.523 | *T* |
| Sequence | 190 | LAITTIYAE | 0.766 | *T* |
| Sequence | 238 | GPIKTKGAF | 0.561 | *T* |
| Sequence | 266 | GRLGTVEEL | 0.916 | *T* |
| | | ^ | | |

Tyrosine predictions

| Name | Pos | Context | Score | Pred |
|---|---|---|---|---|
| | | v | | |
| Sequence | 192 | ITTIYAETG | 0.763 | *Y* |
| | | ^ | | |

Figure 3. Prediction of phosphorylation sites




E and yellow arrows indicate β-strands, and α-helices are shown by H and green columns. C indicates possibility of random coils.

Figure 4. Predicted secondary structure of pig DECR1

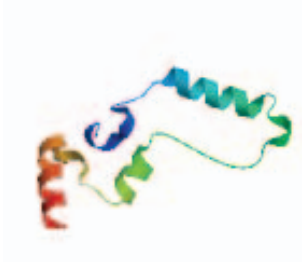


Figure 5. Three-dimensional structure of pig DECR1 protein

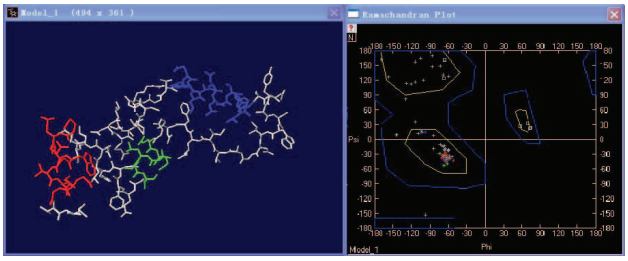


Figure 6. Ramachandran Plot of pig DECR1

## IV. CONCLUSION

In conclusion, the full-length cDNA of DECR1 gene from Mashen pig liver was cloned by RT-PCR and RACE, and bioinformatics analysis as then conducted to predicted the structure and function of DECR1 protein. Although the details of DECR1 function are still unclear, its sequence characterizations provides some new clues not only for further studying the biological functions, but also for regulation of DECR1 on fatty acid metabolism in pig through genetic engineering of DECR1.

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