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An obesity-like gene *MdTLP7* from apple (*Malus* × *domestica*) enhances abiotic stress tolerance

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ABSTRACT

Tubby-like proteins (TLPs) are found in a broad range of multicellular organisms. In mammals, genetic mutation of tubby or other TLPs can result in certain disease phenotypes related to animal specific characters: obesity, retinal degeneration, hearing loss, et al. Plants also harbor a large number of *TLP* genes, but the information in plants is far more limited. We identified a highly up-regulated obesity-like gene, *MdTLP7*, in our previous study of apple differential gene expression profile under chilling, indicating its possible role in plant abiotic stress tolerance. cDNA of *MdTLP7* was amplified and expressed in *Escherichia coli*. In the solid and solution medium, the rate of growth and the quantity of the cell carrying *MdTLP7* gene were significantly more than that of empty vector under salt and temperature stresses. To identify the functional region, serial deletion from both N-terminus and C-terminus of MdTLP7 was performed. In 415 amino acid polypeptide chain of MdTLP7, a middle conservative fragment (120–310 amino acid residues) played vital roles in stress tolerance. This fragment was involved in β barrel of Tubby domain according to the model of Tubby domain. All above results suggested *MdTLP7* confers stress-tolerance to *E. coli* cell against abiotic stresses.

1. Introduction

Plants are generally sensitive to environmental stresses such as high salinity, drought, high temperature, chilling and intense light. These environmental stresses are the primary limiting factors for plant growth and distribution [1]. Facing multifarious environmental stresses, plants adapt themselves through an array of physiological and biochemical changes.

Transcriptome analysis has provided abundant resource for screening new anti-stress genes and valuable insight towards the understanding of molecular mechanism of stress tolerance. Some stress-tolerance genes and their related signaling pathways that control the key processes of plant's responses to stresses have been identified in plants by analyze the change of transcriptome [2,3]. Previously, we developed the differential gene expression profile of apple being subjected to cold stress (unpublished data in our lab). Among significantly up-regulated genes, we identified a gene coding tubby-like protein (TLP), *MdTLP7*, which up-regulated more than 1000 times after being induced by low temperature.

The *Tubby* gene is first identified in obese mouse and its mutation can result in obesity (from which the name "tubby" is derived), loss of vision and hearing, infertility and insulin resistance [4–7]. Since then, a wide array of functions of the gene has been postulated. In a molecular level, Tubby and TLPs has been shown to involve in vesicular trafficking [8], mediation of insulin signaling [9], gene transcription [10], G-protein signalling [11], and ribosomal RNA synthesis [12], among others. TLPs have been discovered in an immense diversity of organisms [13,14]. While four members of Tubby family have already known in mammals [15], plants harbour a large number of TLPs; for instance, 11 members in *Arabidopsis* [13], and 14 members in rice [16,17]. In addition to the typical 270 amino acids conservative C-terminal Tubby domain, plant TLPs have evolved with F-box conservative at the N-terminal sequence, which is otherwise highly divergent in animal. While a wide array of molecular functions of TLPs have been established in animals [8], their role in plants is still elusive. Nevertheless, the highly conservative evolution of tubby proteins and the existence of redundancy suggest their indispensable role in plants. In recent years, this protein family has been shown to be involved in ABA-dependent signaling in *Arabidopsis* [13] and

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pathostress response in rice [16]. The mode of action of tubby family proteins in plants is believed to be regulated by ABA [13], which is characteristically implicated in different stress responses.

We report here the function verification of an obesity-like gene from apple, *MdTLP7*, and identify its functional region. The in vivo functional validation in *Escherichia coli* indicates that this gene is a potential candidate for resistance to salinity and extreme temperature in plants. To our knowledge, this is the first report of *TLP7* involved in abiotic stress response. Studies on *TLP7* provide significant knowledge to reveal the function of *TLPs* in plants.

2. Materials and methods

2.1. Vector construction of full-length and various truncations of MdTLP7

The cDNA full-length and four deletion mutations of *MdTLP7* were amplified from apple cDNA library and inserted in-frame into pET-30a(+) expression plasmid (Novagen) utilizing *EcoRI* and *SalI* restriction sites engineered at 5′- and 3′-ends, respectively to yield recombinant vectors of full-length and four mutations. The recombinant plasmid were sequenced by Sunny biotechnology company (Shanghai China) and confirmed through alignment with the sequence in NCBI, and the recombinant vectors were transformed into *E. coli* Rosetta cell.

2.2. Cell growth under salt and temperature stress on solid and solution medium

Survival test on LB solid medium was carried out to ascertain the function of *MdTLP7* in *E. coli*. *E. coli* cells were transformed with full-length recombinant vector and empty vector, respectively. The cells were grown in LB solution medium to an OD600 of 0.4–0.6 at 37 °C and expression of the recombinant proteins were induced for 2 h with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 37 °C. After the inducement, cultures were diluted to 0.6 OD600, and then diluted to 10^{-2}, 10^{-3} and 10^{-4}. For salt treatments, 10 μl cultures from each dilution were spotted on LB medium supplemented with 0.5 M NaCl or 0.5 M KCl and then incubated at 37 °C for 16 h. While for temperature stress treatments, 10 μl cultures on LB medium were put at 4 °C for 12 h or 50 °C for 1 h, respectively and then incubated at 37 °C for 12 h. The colony number on each plate was counted after incubation.

Growth analysis in LB solution culture was used to determine the region responsible for the stresses. *E. coli* cells with full-length recombinant vector, four mutation recombinant vectors and empty vector, respectively, were grown as mentioned above, diluted to 0.6 OD600 and 20 μl cultures were inoculated in 10 ml LB solution medium containing a high concentration of 0.5 M NaCl or 0.5 M KCl, and incubated at 37 °C on a rotary shaker (150 rpm). The bacterial suspension was harvested at every 2 h till 12 h and OD600 was measured.

All data were duplicated in at least there independent experiments with consistent results.

2.3. Sequence alignment

Multiple sequence alignment was performed with DNAMAN.

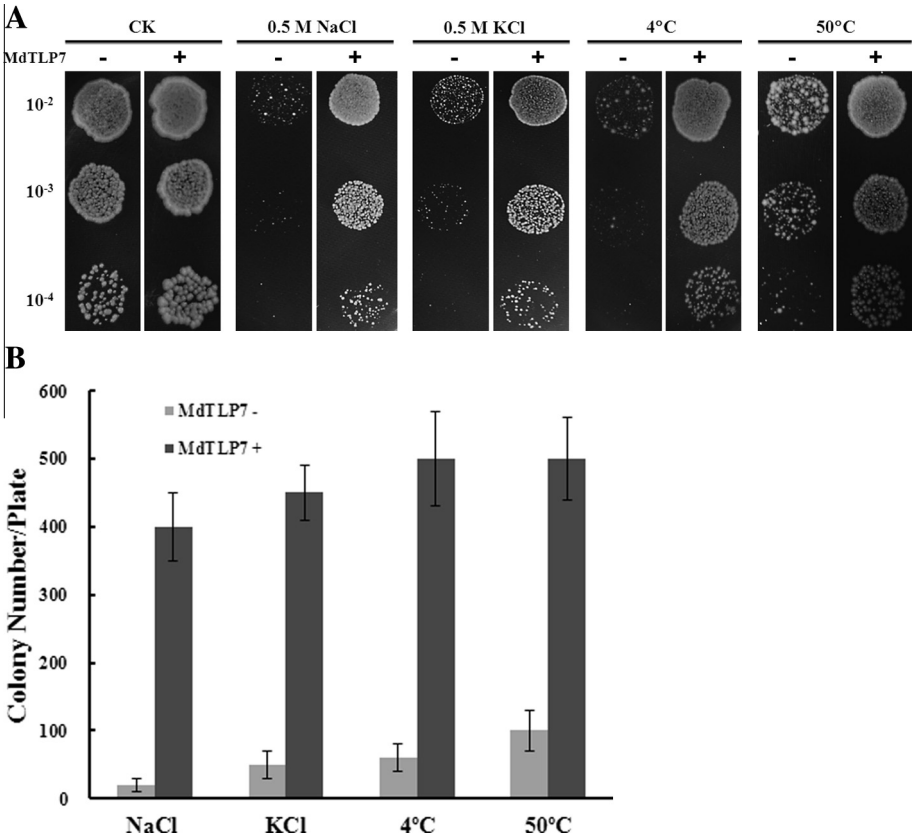

Fig. 1. Survival test of *E. coli* cell carrying *MdTLP7* (+) or empty vector (–) on virous stress conditions. (A) 10 μl cultures from 10^{-2} to 10^{-4} dilutions were spotted on LB basal plates (CK) and plates treated with NaCl, KCl, 4 and 50 °C. (B) The colony numbers appearing on each plate with different treatment were counted.

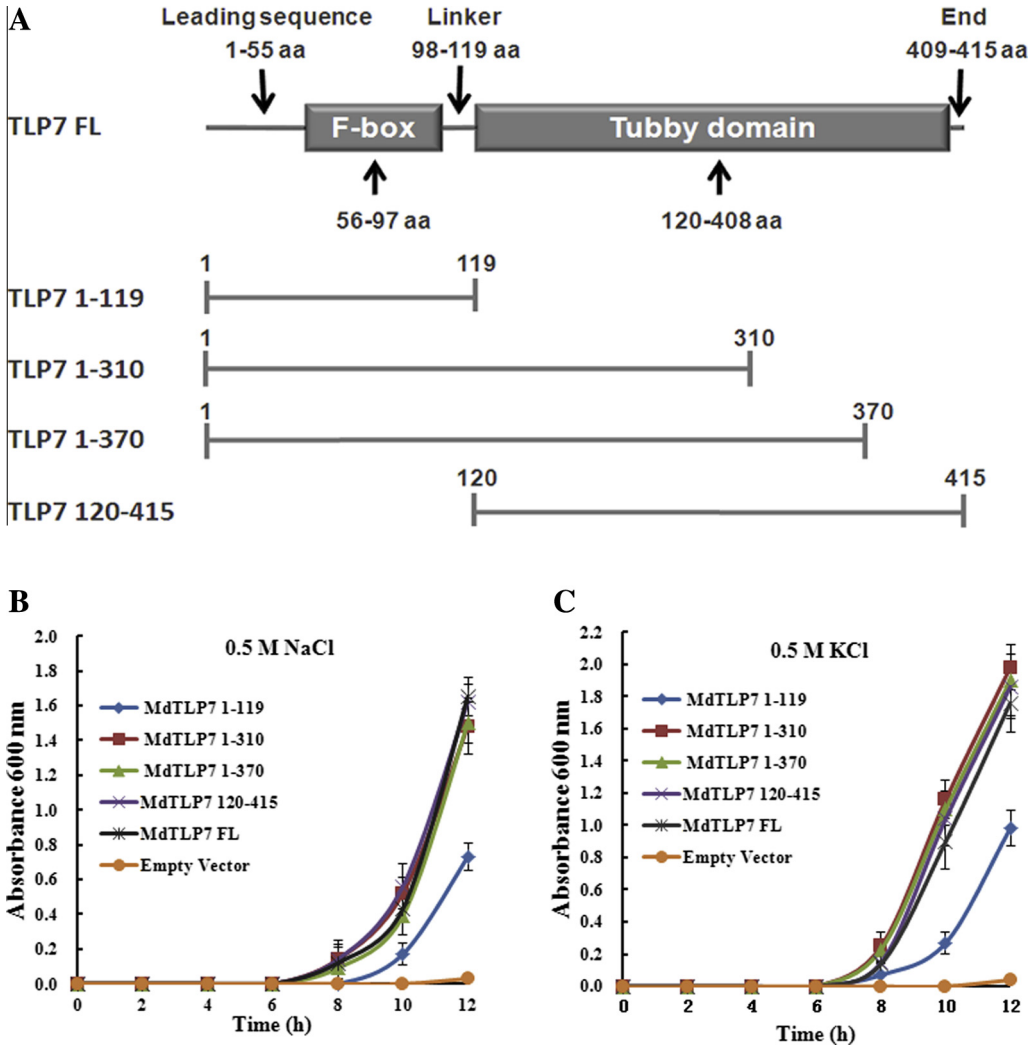

Fig. 2. Growth analysis of *E. coli* cell carrying full length and various truncations of MdTLP7. (A) The various segments of MdTLP7 (MdTLP 1–119, 1–310, 1–370, 120–415) used in the assay were shown schematically with their amino acid positions. (B and C) *E. coli* cell was cultivated in LB solution medium supplemented with 0.5 M NaCl (B), 0.5 M KCl (C). OD600 was recorded at 2 h interval up to 12 h and mean values are represented in graph.

2.4. Homology modeling of tubby domain

The three-dimensional structure of Tubby domain was obtained by homology modeling using the website SWISS-MODEL (http://www.expasy.org/tools).

3. Result and discussion

3.1. Isolation and characterization of MdTLP7

We had previously developed differential gene expression profile of apple under chilling and identified an obesity-like gene, *TLP7,* which was up-regulated more than 1000 times, indicating its possible role in plant abiotic stress tolerance. cDNA of this gene was cloned from the apple cDNA library. It is 1245-bp long and register as HM122708.1 in GenBank. Tubby-like 7 protein has 415 amino acids and consists of a short leading sequence of 55 amino acids followed by an F-box domain, a short 22 amino acid linker and the C-terminal Tubby domain (Fig. 2A).

To gain insight into the structural features of MdTLP7, a molecular model of the tubby domain (120–408 residues) was constructed based on the known crystal structures of TLP2 from *Arabidopsis Thaliana* (At5g01750). Tubby domian folds into a typical structure consists of a β barrel enclosing a central α helix (Fig. 3).

3.2. Expression of MdTLP7 in E. coli enhances growth during abiotic stresses

To ascertain the stress-resistant function of *MdTLP7*, survival test on solid medium was carried out under a variety of stress conditions. Cultures of *MdTLP7* or empty vector transformed *E. coli* were spotted on LB plates for 0.5 M NaCl, 0.5 M KCl, chilling (4 °C) and heat shock (50 °C) treatments. The quantity of the *E. coli* cell carrying *MdTLP7* gene was approximately the same as that of the control empty vector under non-stress conditions as shown in Fig. 1A. In contrast, in the presence of NaCl, KCl, chilling and heat shock, at 10^{-2}, 10^{-3} and 10^{-4} dilutions, the quantity of the cell carrying *MdTLP7* gene was significantly more than that of empty vector (Fig. 1A). The quantity of colonies on each plate with various treatments was counted. The result showed that the colony numbers of recombinants carrying *MdTLP7* were 5–20-fold higher than those of the control strain carrying empty vector under different stresses (Fig. 1B). To evaluate the tolerance capability of *MdTLP7* gene, the viability of the *MdTLP7* transformed *E. coli* in the LB solution mediums supplemented with 0.5 M NaCl and 0.5 M KCl was

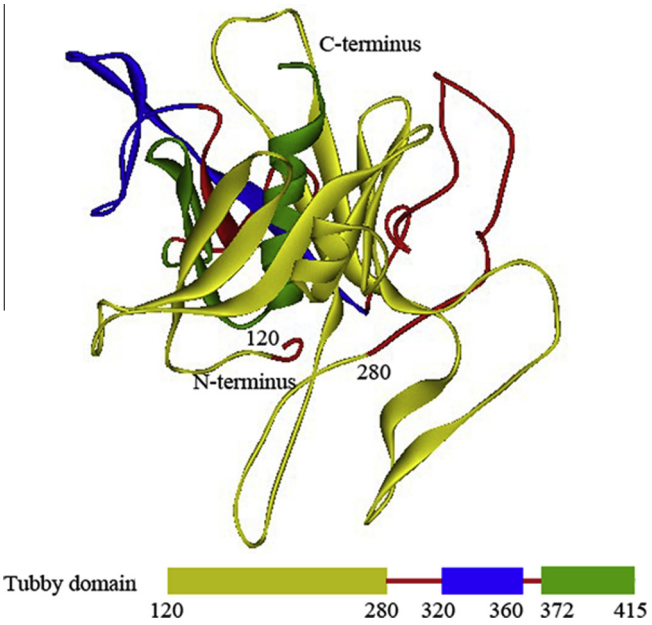

Fig. 3. Homology model of the Tubby domain of MdTLP7. The structure consists of a 12-stranded β barrel filled by a central hydrophobic helix.

examined. The cells with *MdTLP7* were grown into logarithmic phase in 8–9 h after inoculation, while after 12 incubation cells without *MdTLP7* still grew little (Fig. 2B and C). The results above revealed that *MdTLP7* confers strong stress-tolerance to *E. coli* cell against abiotic stresses.

3.3. The β barrel of the Tubby domain played crucial roles in stress tolerance

Growth analysis in solution medium was used to determine the region responsible for the stresses. A series of C- and N-terminal deletion mutations of MdTLP7 protein was generated to test their OD600 compared to the MdTLP7 full length. By the amino acid sequence alignment of MdTLP7 to other MdTLPs family members and all the 11 AtTLPs members, we identified three conservative regions (120–280, 320–360, 372–415 residues) in the Tubby domain and these regions was showed in the three-dimensional model of this domain (Supplementary Fig. 1 and Fig. 3). To determine the functional region, we truncated successively the C-terminal segments to generate the deletion mutations of 1–119, 1–310, 1–370 and the N-terminal segment to generate 120–415 of MdTLP7 (Fig. 2A). The result showed that deletion of 120–310 residues caused MdTLP7 protein lose approximate half of the anti-stress function, suggesting the middle conservative fragment of MdTLP7 played vital roles in stress tolerance. The MdTLP7 1–370, 1–310 and 120–415 transformants still retained almost whole function of full length in both NaCl and KCl stresses (Fig. 2B and C), suggesting that the two conservative regions (320–360, 372–415 residues) in C-terminal and the region preceding the Tubby domain containing the F-box domain (1–119 residues) in the N-terminal are not essential for anti-stress function.

The result of homology modeling showed that the Tubby domain structured by a β barrels and intermediate α helix (Fig. 3). The anti-stress indispensable region (120–280 residues) formed the 8 β strands of β barrel, indicating that the β barrel of the Tubby domain played crucial roles in stress tolerance. The conservative domain in C-terminal (320–415 residues) consisted of two β strands and a α helix, suggesting that the central hydrophobic helix may be maintain the spatial structure, but not essential for the anti-stress function.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.02.005.

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