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RESEARCH ARTICLE

Expression and Construction of Yeast Expression Vector Containing *CsTCTP1* Gene from Cucumber in Yeast Two-Hybrid System

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Abstract:

Background:

The translationally controlled tumor protein (TCTP) was originally found in tumor tissue, and later found in other tissues. Initially, TCTP was considered a kind of growth-associated protein. Recent studies have shown that TCTP has many biological functions.

Objective:

To verification of *CsTCTP1* gene function by yeast two-hybrid system, the pGBKT7- *CsTCTP1* yeast expression vector was constructed and cytotoxicity and self-activating activity were detected, which could lay the foundation for further studies on gene function and make a preparation for verification of *CsTCTP1* gene function by yeast two-hybrid system.

Method:

Specific PCR, conventional sequencing, heat shock conversion method and TE/LiAC transformation method.

Results:

We constructed a yeast expression vector containing the *CsTCTP1* gene. The *CsTCTP1* coding sequence was inserted into a pGBKT7 vector as a bait protein and then transformed into the Y2HGold yeast stain.

Conclusion:

We found that CsTCTP1 protein had no cytotoxic effect and could not be self-activated. The constructed bait expression vector can be used in the subsequent yeast two - hybrid detection system.

Keywords: TCTP, PGBKT7 bait vector, Yeast two-hybrid, Y2HGold, Cytotoxic, Self-activated.

INTRODUCTION

The translationally controlled tumor protein (TCTP) is a highly conserved protein that is abundant in plant and animal cells and has different functions. The TCTP was initially identified in mouse tumor cells [1, 2]. Studies indicated that TCTP has a variety of biological functions: calcium binding protein (CBP) [3], histamine release factor [4], tubulin binding protein [5], anti-apoptotic protein [6, 7], anti-oxidation [8], involved in stress response and signal transduction [9, 10] and so on.

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In plants, research about TCTP protein function is still scarce. However, the similarity of TCTP structure of plantsand animals suggested that they may have similar functions. Plant TCTP proteins play key roles in both abiotic and biotic stress. It was reported that TCTP transcript or protein levels responded to different stress conditions like drought, aphids, cold, high temperature, salt, light, water deficit and so on [11 - 16].

Powdery mildew, caused by *Sphaerotheca fuliginea* (Sf), is a widely distributed and destructive disease of greenhouse and field-grown cucumber plants and causes great yield loss. In a previous study, TCTP showed increased abundance in powdery mildew resistant line B21-a-2-1-2 (highly resistant) by 2-DE [17].

Protein - protein interactions is an important way to reveal the new role of the proteins. Yeast two-hybrid is a common method for protein interaction screening. Y2HGold yeast stain is a common stain that was used in Yeast two-hybrid system, Y2HGold was constructed of four reporter genes--ADE2, AUR1-C, HIS3 and MEL1 in GAL4 UASs (GAL4 upstream activating sequences) and downstream of the promoter through genetic engineering. GAL4 can specially bind with promoter and activate the downstream reporter gene expression. In this study, the coding sequence of *CsTCTP1* was amplified. The PCR product was cloned into the pGBKT7 vector to generate pGBKT7-*CsTCTP1*, and then the bait vector was transformed into Y2HGold yeast stain. Cytotoxicity and self-activation were detected. On this basis, we can further screen proteins interacting with cucumber TCTP protein by yeast two-hybrid system, building networks of protein interactions, for understanding its role in the cell.

MATERIALS AND METHODS

Plant Materials Handling and Growth Conditions

Cucumber seedlings were Jinyan 4th. Cucumber seeds were sterilized with 50% NaClO 15min and 75% ethyl alcohol for 45s. The sterilized seeds were germinated on a solid MS medium in a growth chamber at 24°C with light intensity of 40 lux. Seedlings with two true leaves were carefully transferred into Soil matrix, and then grown in a greenhouse.

Specific Primer Design and Amplification of *CsTCTP1* Gene Sequence

Primer pairs were designed using Primer 5.0 software and synthesized by Shanghai Biological Engineering Co., Ltd. The primers used in this study are listed in Table **1**.

Table 1. CsTCTP1 gene primer sequences, (5' to 3'). Represented restriction sites are underlined.

Gene name	Primer sequence (5' to 3 ')
CsTCTP1-F	CGAATTCATGTTGGTTTATCAGGACC
CsTCTP1-R	CGGATCCTCAGCACTTGACTTC

Total RNA was isolated from the cucumber leaves using RNA plant plus kit (Tiangen). Complementary DNA (cDNA) was synthesized using oliego (dT) primers (Takara) and PrimeScript^{TM} RTase reverse transcriptase (Takara) for 1 h at 42°C. Resulting cDNA were used for amplified *CsTCTP1* coding sequence. The PCR product was purified using DNA Gel Extraction kit.

The Bait Vector Construction and Transformation

To obtain the pGBKT7-*CsTCTP1* bait vector, the pMD18-T-*CsTCTP1* vector was digested with *Eco*RIand *Bam*HI, the pGBKT7 plasmid was also digested with *Eco*RIand *Bam*HI. Then, the connection of *CsTCTP1* and pGBKT7 was executed using T_4 DNA ligase.

Transformation of Y2HGold and Self-Activation Detection

The TE/LiAC method [18] was used for preparing Y2HGold competent yeast cells, and the recombinant plasmid was transformed into Y2HGold Yeast stain through Lithium acetate transformation method. The conversion solution was incubated in SD/-Trp, SD/-Trp-Leu and SD/-Trp-His-Ade medium respectively. The positive clones were detected by colony PCR.

RESULTS

Amplification of *CsTCTP1*

CsTCTP1 gene PCR amplification products and cycle products were detected by 1% agarose gel electrophoresis which showed that the specific fragment was about 500 bp, consistent with that expected (Fig. **1**).

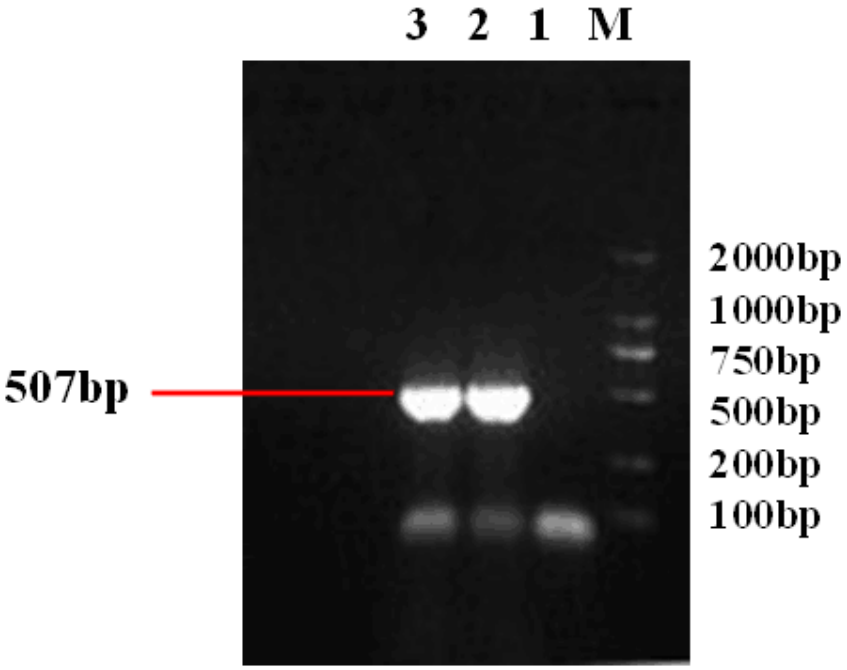

Fig. (1). Electrophoresis profile of PCR products of CsTCTP1 gene M: DNA marker DL2000; 1: Water; 2, 3: *CsTCTP1* PCR amplified product.

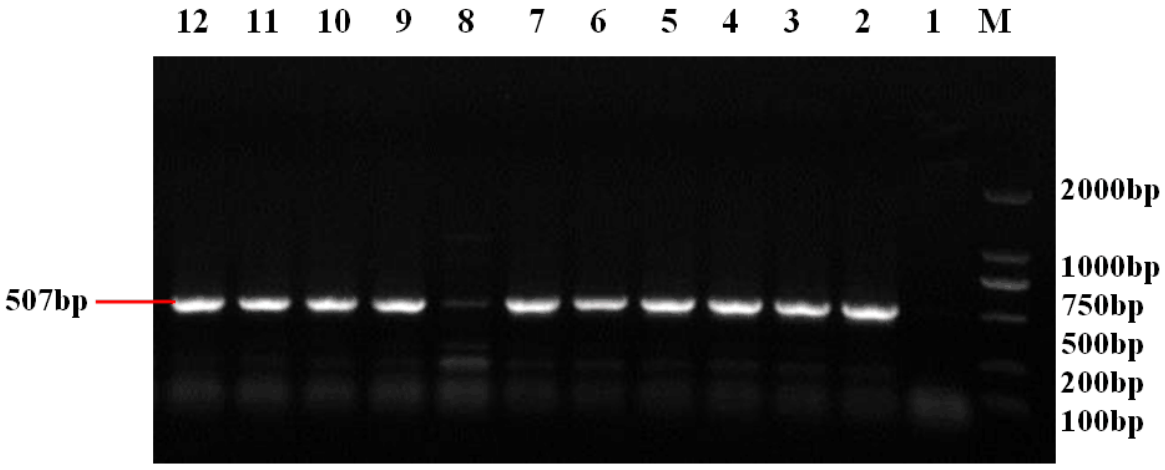

Fig. (2). Colony PCR of recombinant plasmid *pGBKT7-CsTCTP1* M: DNA marker DL2000; 1: Water; 2-12: Colony PCR products of *CsTCTP1* gene.

Construction of Bait Expression Vector

To construct the recombinant bait vector, we recycle the small fragments of pMD18-T-*CsTCTP1* and large fragments of pGBKT7 plasmid after double digest with EcoR I and BamH I. The colony PCR products of recombinant

plasmid pGBKT7-*CsTCTP1via* agarose gel electrophoresis analysis showed 507 bp of the target gene band (Fig. **2**). Sequencing results showed that the gene fragments was consistent with the target gene sequence in GenBank (Fig. **3**).

Fig. (3). Sequencing results recombinant vector pGBKT7-*CsTCTP1*.

Cytotoxic and Self-activation Detection

To detect whether CsTCTP1 protein has cytotoxic or self-activation, we transformed pGBKT7-*CsTCTP1* bait expression vector into Y2HGold yeast stain, and incubated it in SD/-Trp, SD/-Trp-Leu and SD/-Trp-His-Ade medium at 30°C. The results showed that pGBKT7-CsTCTP1 and pGBKT7 stain could grow on SD/-Trp medium. The yeast growth curve of pGBKT7-CsTCTP1 transformants was the same as pGBKT7 transformants. Both of transformants could not grow on SD/-Trp-Leu and SD/-Trp-His-Ade medium, which indicated that CsTCTP1 protein had no cytotoxic and no self-activation (Figs. **4**, **5**) which could lay the foundation for screening CsTCTP1 interacting target proteins using the yeast two-hybrid.

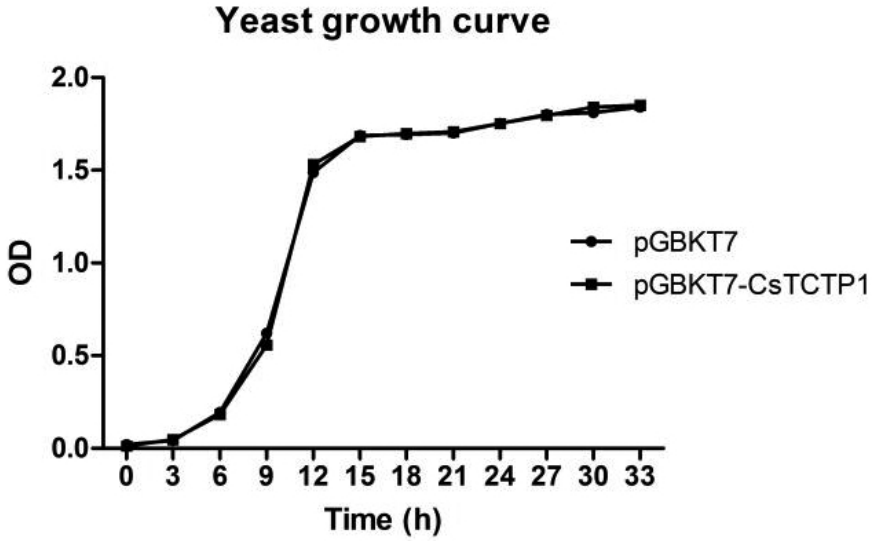

Fig. (4). Yeast growth curve of *pGBKT7-CsTCTP1* and pGBKT7 transformants.

DISCUSSION

As previously mentioned, although TCTP has many biological functions, its interaction mechanism is unclear, particularly in plants. TCTP gene function analysis of *Arabidopsis* from the pros and cons, both proved TCTP was related to plant growth, and after the *Drosophila* TCTP gene was transferred to transgenic *Arabidopsis* where TCTP had been knocked out, as it can restore growth [19]. Studies have shown that TCTP could interact with TSC-22 and p53 and affect the stability of these two proteins. Overexpression of TCTP could promote the degradation of TSC-22, thereby inhibiting apoptosis mediated by TSC [20]. Human hTCTP gene could interact with HRheb in vitro and promote hRheb to release GDP. hTCTP can also improve mTORC1 activity in vivo [21]. TCTP can also increase NADPH-dependent reactive oxygen species (ROS), and stimulate the cytoskeleton and cell movement [22].

Brioudes *et al.* conducted a positive and negative identification of the TCTP gene of *Arabidopsis thaliana*. It was proved that TCTP was related to growth, and the TCTP gene of *Drosophila* was transformed into the transgenic *Arabidopsis thaliana* knocked out of TCTP gene, its growth rate can be restored, indicating that TCTP gene has the same functions both in plants and animals [19]. A recent study showed that tobacco TCTP protein could interact with ethylene receptor tobacco histidine kinase1 enhancing plant growth through promotion of cell proliferation. Our study found that cucumber TCTP may have important effect on powdery mildew resistance. We are able to detect whether cucumber TCTP protein could interact with other protein to exercise its functions. It has been found that the constructed bait expression vector has no toxicity and self-activating activity and can be used in the further experiments, which would lay a foundation for further studies of *TCTP* gene function and protein interaction mechanism in plants and animals.

Protein - protein interactions is an important way to study protein function, yeast two-hybrid protein interaction studies is a common method. In this work, the three-reporter gene was used to verify the cytotoxic effect and self-activation of fusion protein in yeast, which induced the false positive in further yeast two hybrid assays. Our study intends to make CsTCTP1 protein as a bait to screen the interaction proteins, and understand how the CsTCTP1 protein exercises their functions.

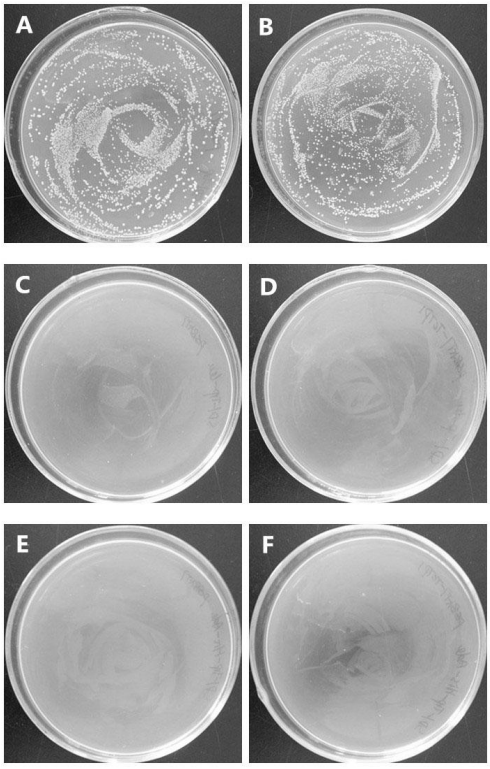

Fig. (5). Cytotoxic and self-activation detection. A, B: pGBKT7-*CsTCTP1* and pGBKT7 transformants incubated at SD/-Trp medium. C, D: pGBKT7-CsTCTP1 and pGBKT7 transformants incubated at SD/-Trp-Leu medium. E, F: pGBKT7-*CsTCTP1* and pGBKT7 transformants incubated at SD/-Trp-His-Ade medium.

CONCLUSION

We have successfully amplified and purified of *CsTCTP1* gene in cucumber coding sequence, and constructed the yeast two-hybrid yeast expression vector. The bait vector was successfully transformed to Y2HGold, which has no cytotoxic effect and no self-activation. Our study is a preparation for screening CsTCTP1 interaction proteins by yeast two-hybrid.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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