Expression and catalytic function analysis of cassava hexokinase gene MeHXK3

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Abstract

Hexokinase is a dual-function enzyme that phosphorylates glucose and fructose to form hexose- phosphate and plays a key role in the process of glucose signal transduction. In this study, the expression of *MeHXK3* gene and the catalytic function of its encoded product were further analyzed. Transcriptional data analysis showed that *MeHXK3* was highly expressed in OES and FEC. The HXK activity of MeHXK3 was identified by functional complementation of the HXK-deficient yeast strain YSH7.4-3C (*hxk1, hxk2, glk1*). The gene expression and catalytic functional identification of *MeHXK3* suggest that it might play an important role for hexose phosphorylation during OES and FEC development.

Keywords

Cassava, hexokinase, yeast complementation.

1. Introduction

In higher plants, the hexoses (glucose and fructose), which obtain from the degradation of sucrose or starch must be phosphorylated to hexose phosphate before further metabolised [1]. Hexokinases (HXKs) can phosphorylate glucose and fructose [2]. It has been reported that HXKs have an important role in plant development and sugar-sensing [3]. Cassava (Manihot esculenta) is the most important root crop in the tropics, which accumulates starch in tuber roots [4]. Studies have shown that HXKs are the key enzyme in cassava root development and starch synthesis [5]. Our laboratory has obtained seven HXK genes (MeHXK1-7) from the South China No.8 (SC8) cassava variety. The expression characteristics of MeHXKs were studied by using qPCR technique. The results showed that MeHXK3 was lowly expressed at tuber roots during the accumulation of starch [6]. Bioinformatics prediction found that MeHXK3 may be a HXK-like (HKL) protein, only has a regulatory function and lacks a catalytic function [6]. In order to elucidate the function of MeHXK3 in cassava plants, the expression of MeHXK3 genes and the catalytic function of its encoded product were further analyzed.

2. Materials and Methods

2.1 Materials

The DH5α strain of Escherichia coli was used for the construction of yeast expression vector. The hexokinase-deficient yeast strain YSH7.4-3C was kindly provided by Prof. Stefan Hohmann (University of Gothenburg).

2.2 Construction of Yeast Expression Recombinant Vector

A yeast shuttle vector, pDR195, containing the URA3 gene as a selective marker was used to express the cDNA of MeHXK3 in YSH7.4-3C yeast cells. The cDNA was inserted as a Xho I/BamH I fragment into the Xho I/BamH I sites within pDR195, and transformed into E. coli to screen the positive clones, and the result vector was verified (sequencing analysis and restriction enzyme digestion) and designated as pDR195- MeHXK3. Yeast transformations were carried out by using lithium acetate method and verified by PCR.

2.3 Yeast Complementation

The YSH7.4-3C yeast cells consisting pDR195 or pDR195-MeHXK3, and YSH7.4-3C yeast cells were grown on YPgal medium consisting 1% yeast extract, 2% Bacto™ peptone, and 2% galactose. The selective medium for the transformed colonies was SD media (no uracil), which contains 2% of a carbon source (D-glucose or D-fructose). The YSH7.4-3C yeast cells were transformed with the pDR195 vector alone, which used as a control.

2.4 Differential Expression Analysis of MeHXK3

For differential expression analysis of MeHXK3, The Illumina RNA-seq data that were previously generated by Wilson et al (Series GSE82279) was utilized [7]. The expression level was measured by fragments per kilobase of exon model per million reads mapped (FPKM) values.

3. Results

3.1 Construction of Yeast Expression Vector pDR195-MeHXK3

The cDNA coding sequence of MeHXK3 gene was obtained by using PCR amplification (Fig. 1). The amplified MeHXK3 gene was digested with Xho I/BamH I, and ligated with pDR195 yeast expression vector; and the result vector was verified by digesting; and designated as pDR195-MeHXK3 (Fig. 2).

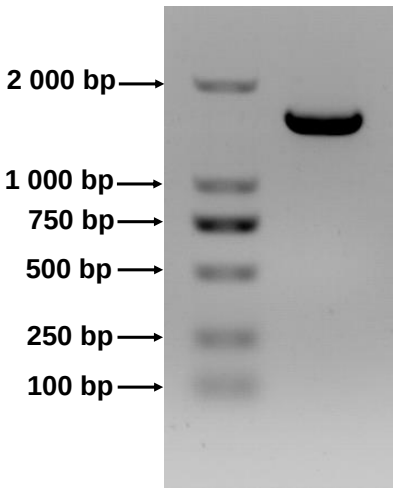

Fig. 1 The electrophoresis for PCR product of MeHXK3 gene M: DNA marker DL2000; 1: PCR product of MeHXK3 gene

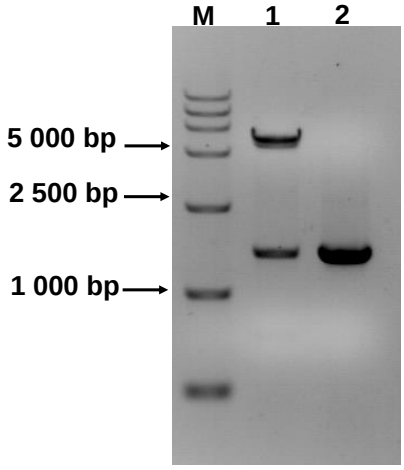

Fig. 2. The electrophoresis of pDR195- MeHXK3 vector digesting by Xho I and BamH I restriction enzymes M, DNA marker DL15000; 2: The electrophoresis of pDR195- MeHXK3 vector cut by Xho I and BamH I restriction enzymes, 2, MeHXK3 gene

3.2 Yeast Transformation

The recombinant vector pDR195-MeHXK3 was transformed into hexokinase-deficient yeast strain YSH7.4-3C; and then grown on SD (lack uracil) medium with D-galactose as carbon source. Six single colonies were selected for PCR reaction. The results of electrophoresis showed that the recombinant vector pDR195-MeHXK3 has been transformed into YSH7.4-3C (Fig. 3).

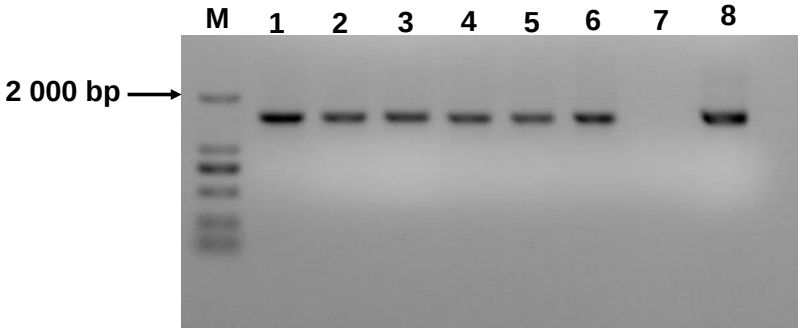

Fig. 3 Identification of yeast by PCR M, Marker DL2000; 1~6, Yeast colony PCR; 7, Negative control; 8, Positive control

3.3 Functional Analysis of MeHXK5 Catalyzing Glucose Phosphorylation

Activity of MeHXK3 for glucose phosphorylation was examined on the selection medium containing glucose. The yeast mutant YSH7.4-3C that lacks endogenous HXK activity, and the YSH7.4-3C carrying the recombinant pDR195-MeHXK3 and the empty pDR195 were tested. The results showed that the yeast mutant YSH7.4-3C, the YSH7.4-3C carrying the empty pDR195 vector could not grow on the medum; and the yeast mutant YSH7.4-3C carryong the recombinant pDR195-MeHXK3 vector could grow on the medium (Fig. 4). This result suggested that MeHXK3 has function to catalytic the phosphorylation of glucose.

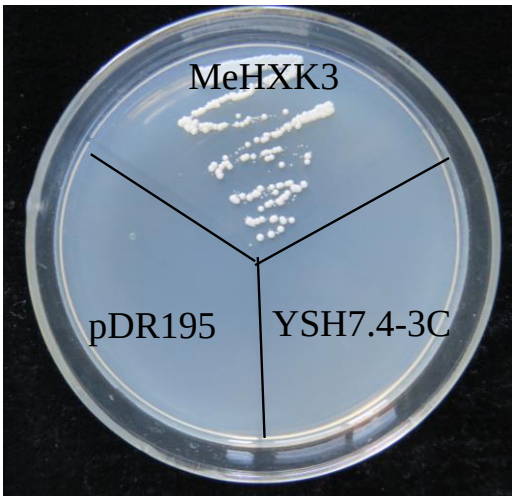

Fig. 4. Growth analysis of the transgenic yeast cells on the SD medium (- Uracil) with glucose as sole carbon source MeHXK3: YSH7.4-3C was transformed with the pDR195- MeHXK3; pDR195: YSH7.4-3C was transformed with the empty vector pDR195; YSH7.4-3C: the HXK mutant YSH7.4-3C. All the yeast cells were grown on the SD medium with glucose as the sole carbon source at 30℃ for 3 d.

3.4 Functional Analysis of MeHXK5 Catalyzing Fructose Phosphorylation

Activity of MeHXK3 for fructose phosphorylation was examined on the selection medium containing fructose. The YSH7.4-3C, the YSH7.4-3C carrying the recombinant pDR195-MeHXK3 and the empty pDR195 vectors were tested. The results showed that the YSH7.4-3C and the YSH7.4-3C carrying the empty pDR195 vector could not grow on the selection medium, whereas the yeast cells YSH7.4-3C carrying the pDR195-MeHXK3 vector could grow on this medium (Fig. 4). This result suggested that MeHXK3 has function to catalytic the phosphorylation of fructose.

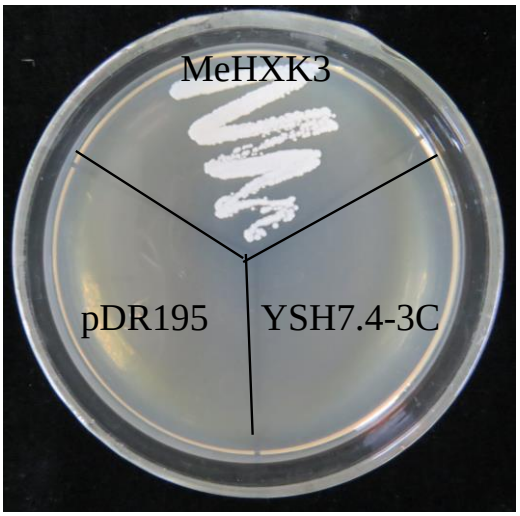

Fig. 5. Growth analysis of the transgenic yeast cells on the SD medium (- Uracil) with fructose as sole carbon source MeHXK3: YSH7.4-3C was transformed with pDR195- MeHXK3; pDR195: YSH7.4-3C was transformed empty vector pDR195; YSH7.4-3C: the HXK mutant YSH7.4-3C. All the yeast cells were grown on SD medium with fructose as the sole carbon source at 30℃ for 3 d.

3.5 Expression pattern analysis of MeHXK3

The tissues/organs expression profiles of MeHXK3 were determined in the leaf (L), midvein (MV), petiole, lateral bud (LB), organised embryogenic structures (OES), friable embryogenic callus (FEC), shoot apical meristem (SAM), root apical meristem (RAM), fibrous root (FR), storage root (SR) of cassava according to the Illumina RNA-seq data. The results showed that MeHXK3 was highly

espressed in OES, then followed by FEC, LB, S, SAM, RAM. The expression of MeHXK3 in L, MV, P, FR, and SR was low.

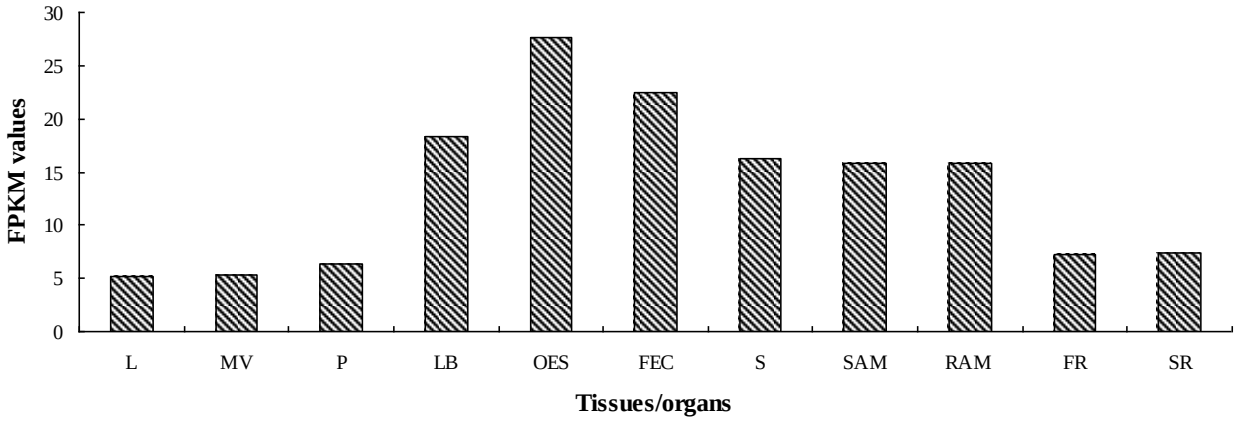

Fig. 6 Expression of the MeHXK3 gene in different tissues/organs of cassava L: leaf; MV, madden; P: petiole; LB: lateral bud; OES: organized embryo genic structures; FEC: friable embryo genic callus; S: stem; SAM: shoot apical meristem; RAM: root apical meristem; FR: fibrous root; SR: storage root.

4. Conclusion

The results of this article indicate that the expression of the MeHXK3 gene in yeast cells has a complement to the hexokinase mutant, suggesting that MeHXK3 has a hexose phosphorylation function. And MeHXK3 is highly expressed in OES and FEC, it suggests that MeHXK3 may play an important role during OES and FEC development.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 31600196, 31671767, 31601359, 31371706), the earmarked fund for China Agriculture Research System (CARS-11-HNGJC).

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