Catalytic functional identification of Alkaline/Neutral invertase MeNINV7 from cassava

Yuan Yao[1, a], Lu Shang[2, b], Yunlin Wang[2, c], Chong Sun[3, d], Xiaohua Lu[2, e], Mengting Geng[2, f], Ruimei Li[1, g], Shaoping fu[1, h], Ruijun Duan[1, i], Jiao Liu[1, j], Xinwen Hu[2, k], and Jianchun Guo[1, l, *]

[1]Key Laboratory of Biology and Genetic Resources of Tropical Crops, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China;

[2]College of Agriculture, Hainan University, Haikou 570228, China;

[3]College of Life Science and Biotechnology, Heilongjiang Bayi Agricultural University, Daqing 163319, China.

[a]yaoyuan@itbb.org.cn, [b]18689606176@163.com, [c]13876830997@163.com, [d]sun780347812@163.com, [e]985428433@qq.com, [f]mengtinggeng8908@163.com, [g]liruimei@itbb.org.cn, [h]fushaoping@ itbb.org.cn, [i]duanruijun@itbb.org.cn, [j]liujiao@itbb.org.cn, [k]huxinwen@hainu.edu.cn, [l,*]Corresponding author: jianchunguoh@163.com

Abstract

The cassava tuber roots are rich in starch. Sucrose provides carbohydrate and energy for the synthesis of cassava starch. Alkaline/Neutral invertase is a key enzyme for hydrolysis the of sucrose. Presently, 11Alkaline/Neutral invertase genes have been cloned from the cassava genome, and the expression of *MeNINV7* was highest in stems. In this study, an invertase mutant yeast strain SEY2102 was used to determine whether MeNINV7 could catalyze the hydrolysis of sucrose . The result found that SEY2102 yeast carrying pDR196-*MeNINV7* vector could be grown on the medium with sucrose as the sole carbon source, and the crude enzymes from these yeast cells have invertase enzymatic activity for sucrose hydrolysis under Alkaline/Neutral pH conditions. Therefore, the MeNINV7 is an Alkaline/Neutral invertase. These results provide a basis for further study of the enzymatic properties and physiological functions of MeNINV7.

Keywords

Cassava, Alkaline/Neutral invertase, catalytic function.

1. Introduction

Cassava tuber roots are rich in starch, and the main food crop in tropical and subtropical regions [1]. Sucrose provides carbohydrates and energy for the synthesis of cassava starch, which must be converted into hexose by invertase or sucrose synthase [2, 3]. The invertase catalyzes the decomposition of sucrose into glucose and fructose [4]; and it can be divided into Acidic invertase and Alkaline/Neutral invertase according to the suitable pH for the decomposition of sucrose [5]. Studies have shown that invertase is a key enzyme in cassava root development and starch synthesis [6]. Our laboratory has obtained 11 Alkaline/Neutral invertase genes (MeNINV1-10, and nINV1) from the South China No.8 (SC8) cassava variety. The expression characteristics of Alkaline/Neutral invertase gene family of cassava were studied by using qPCR technique. The results showed that MeNINV7 was expressed in the leaves, stems, flowers, fruits, root phloem and root xylem of cassava, while the highest expression was in stems. Starch can be synthesized and accumulated in the cassava stems, these starches may participate in responding to stress [7]. Therefore, it is speculated that MeNINV7 plays an important role in sucrose decomposition and starch storage in cassava stems. In order to further verify whether MeNINV7 has the function of catalyzing the decomposition of sucrose,

MeNINV7 is linked to pDR196 yeast expression vector and transferred into invertase mutant yeast strain SEY2102 for functional complementation experiment. This study will lay a foundation for further analysis of the physiological functions of MeNINV7 in sucrose decomposition and starch storage in cassava stems.

2. Materials and Methods

2.1 Materials

The DH5α strain of Escherichia coli used in this experiment was stored in our laboratory. The invertase mutant yeast strain SEY2102 was kindly provided by Jie Liu (Northwest A&F University).

2.2 Construction of Yeast Expression Recombinant Vector

A yeast shuttle vector, pDR196, containing the URA3 gene as a selective marker was used to express the cDNA of MeNINV7 in SEY2102 yeast cells. The cDNA was inserted as a Pst I/Sal I fragment into the Pst I/Sal I sites within pDR196, and transformed into E. coli. The result vector was verified (sequencing analysis and restriction enzyme digestion) and designated as pDR196-MeNINV7. Yeast transformations were carried out by using lithium acetate method and verified by PCR.

2.3 Yeast Complementation

The SEY2102 yeast cells containing the pDR196 or pDR196-MeNINV7 were grown on YPD medium. The selective media for uracil auxotrophic growth of the transformed colonies were SD media (lacking uracil) containing 2% of either sucrose or fructose as a sole carbon source. The yeast cells were grown on the selective media for 3 days under 30 °C to observe its growth. The SEY2102 yeast cells without any transformed vector and the SEY2102 transformed with pDR196 vector served as the controls.

2.4 Identify Enzymatic Activity of MeNINV7

The SEY2102 yeast cells transformed with either pDR196 or pDR196-MeNINV7, or without any transformation were harvested and broken by vortexing with glass beads at 4°C. Lysates were centrifuged and the supernatant was used as a crude enzyme to identify the Alkaline/Neutral invertase activity by measure reducing sugars using the 3,5-dini-trosalicylic acid (DNS) method according to Jie Liu et al [8].

3. Results

3.1 Construction of Yeast Expression Vector pDR196-MeNINV7

In order to construct yeast expression vector, the pMD18T-MeNINV7 plasmid was used as template to carry out PCR amplification with primers (content restriction sites Pst I and Sal I) to obtain the cDNA coding region of MeNINV7 gene. The results of electrophoresis showed that the size of the amplified fragment was consistent with the expected size, and the strip is single (Fig. 1). The amplified MeNINV7 gene was recovered and digested with Pst I and Sal I, and ligated with pDR196 yeast expression vector. The correct clones were selected for extraction of the recombinant plasmid, which were identified by enzyme digestion. The results of electrophoresis showed that a band of about 2049 bp was obtained (Fig. 2), which was consistent with the size of MeNINV7 gene. The recombinant plasmid pDR196-MeNINV7 has been successfully constructed.

3.2 Yeast Transformation

The recombinant plasmid pDR196-MeNINV7 was transformed into invertase mutant yeast strain SEY2102 and then grown on SD (lack uracil) medium and fructose as sole carbon source. Eight single colonies were selected for PCR reaction. The results of electrophoresis showed that the recombinant plasmid pDR196-MeNIINV7 had been transformed into SEY2102 (Fig. 3).

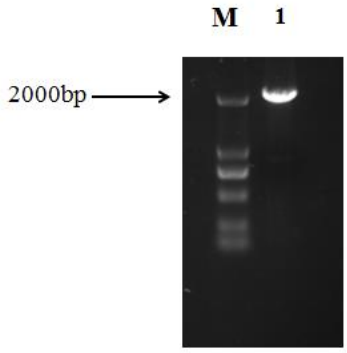

Fig. 1 The electrophoresis for PCR product of MeNINV7 geneM: DNA marker DL2000; 1: PCR product of MeNINV7 gene

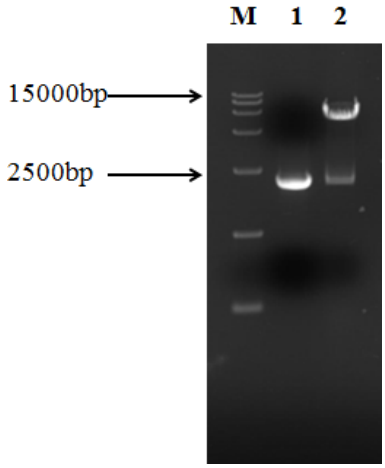

Fig. 2 The electrophoresis of pDR196- MeNINV7 vector digesting by Pst I and Sal I restriction enzymes

M: DNA marker DL15000; 1: *MeNINV7* gene; 2: The electrophoresis of pDR196- *MeNINV7* vector cut by *Pst* I and *Sal* I restriction enzymes

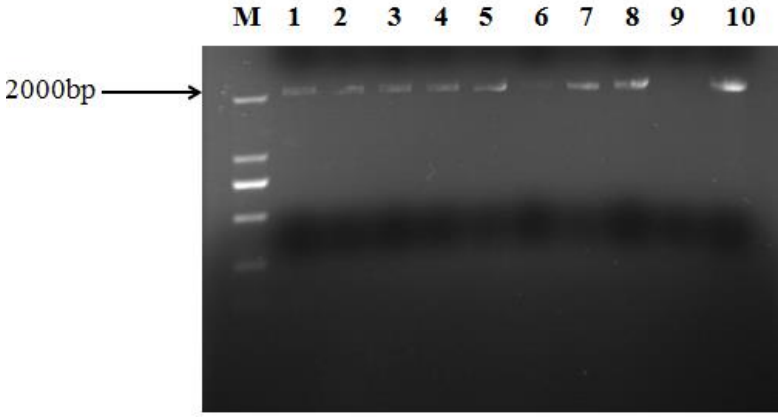

Fig. 3 Identification of yeast by PCRM: Marker DL2000; 1~8: Yeast colony PCR; 9: Positive control; 10: Negative control

3.3 Functional Analysis of MeNINV7 by Yeast Complementary

The activity of MeNINV7 was examined in the yeast mutant SEY2102, the SEY2102 carrying the empty pDR196 vector, and the recombinant pDR196-MeNINV7, respectively. The results showed that SEY2102 and SEY2102 carrying the empty pDR196 vector could not grow on the selection medium containing sucrose, as they lacked endogenous invertase activity and is unable to grow on

sucrose as a sole carbon source; whereas the yeast cells carrying the pDR196-MeNINV7 could grow on this media (Fig. 4A). All the yeast cells SEY2102, and SEY2102 carrying the empty pDR196 or pDR196-MeNINV7 vectors could grow on the medium containing fructose (Fig. 4B). This result suggested that MeNINV7 has invertase function to catalytic the decomposition of sucrose.

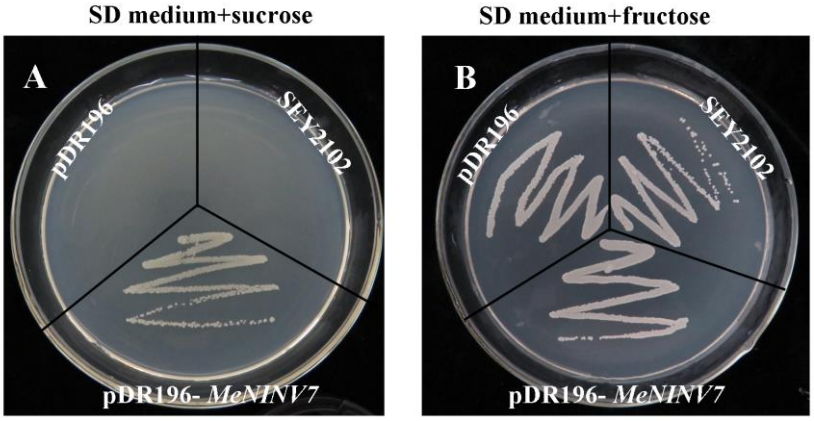

Fig. 4 Invertase activity analysis of the transgenic yeast cells

pDR196-MeNINV7: the SEY2102 was transformed with pDR196- MeNINV7; pDR196: the SEY2102 was transformed with empty vector pDR196; SEY2102: the invertase mutant SEY2102; The Yeast cells were grown on SD medium with sucrose (A) or fructose (B) as the sole carbon sources at 30℃ for 3 d.

3.4 Alkaline/Neutral Invertase Enzyme Assay of MeNINV7

The crude enzyme from SEY2102 yeast cells and the transformed yeast cells containing pDR196-MeNINV7 or pDR196 vectors were extracted; and identified their Alkaline/Neutral invertase activity at pH 7.5.. Generally, Sucrose was decomposed into hexose by Alkaline/Neutral invertase; and the hexose could react with the DNS reagent to form a reddish brown substance. The results showed that the crude enzyme from the SEY2102 and the SEY2102 carrying the empty pDR196 vector had no enzymatic activity; whereas the crude enzyme from the SEY2102 carrying pDR196-MeNINV7 vector had Alkaline/Neutral enzymatic activity. This result indicates that MeNINV7 catalyzes the decomposition of sucrose under Alkaline/Neutral pH conditions.

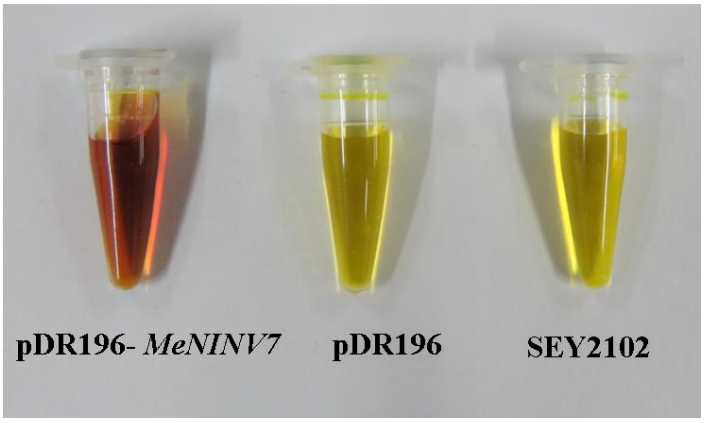

Fig. 5 Identify enzymatic activity of MeNIINV4 by DNS reaction

pDR196-MeNINV7: the SEY2102 was transformed with pDR196- MeNINV7; pDR196: the SEY2102 was transformed empty vector pDR196; SEY2102: the invertase mutant SEY2102

4. Conclusion

The results of this article indicate that the expression of the MeNINV7 gene in yeast cells have a complement to the invertase mutant; and MeNINV7 protein could hydrolyze sucrose into hexose at pH 7.5. Therefore, MeNINV7 is an Alkaline/Neutral invertase.

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).

References

[1] M.A. El-Sharkawy. Cassava biology and physiology, Plant molecular biology, vol. 53. (2003), 621-641.
[2] Y.Z. Li, J.Y. Zhao, S.M. Wu, et al. Characters related to higher starch accumulation in cassava storage roots, Scientific Reports, vol. 6. (2016), 19823.
[3] Y. Baguma, C. Sun, M. Borén, et al. Sugar-mediated semidian oscillation of gene expression in the cassava storage root regulates starch synthesis, Plant Signaling & Behavior, vol. 3. (2008), 439.
[4] J. Siemens, M.C. GonzáLez, S. Wolf, et al. Extracellular invertase is involved in the regulation of clubroot disease in Arabidopsis thaliana, Molecular plant pathology, vol. 12 (2011), 247-262.
[5] T. Roitsch, M.C. González. Function and regulation of plant invertases: Sweet sensations, Trends in Plant Science, vol. 9. (2004), 606-613.
[6] C. Jansson, A. Westerbergh, J. Zhang, et al. Cassava, a potential biofuel crop in (the) people's republic of china, Applied Energy, vol. 86, Supplement 1. (2009), S95-S99.
[7] Y. Yao, M.T. Geng, X.H. Wu, et al. Genome-wide identification, expression, and activity analysis of Alkaline/Neutral invertase gene family from cassava (Manihot esculenta Crantz), Plant Molecular Biology Reporter, vol. 33. (2015), 304-315.
[8] J. Liu, L. Han, B. Huai, et al. Down-regulation of a wheat Alkaline/Neutral invertase correlates with reduced host susceptibility to wheat stripe rust caused by Puccinia striiformis, Journal of Experimental Botany, vol. 66. (2015), 7325-7338.