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Role of *SUC2* gene and invertase of *Saccharomyces* sp. W0 in inulin hydrolysis

Yang Li, Wen-Juan Fu, Nan-Nan Liu, Mei-Juan Tan, Guang-Lei Liu, Zhen-Ming Chi*

UNESCO Chinese Center of Marine Biotechnology and Institute of Evolution and Marine Biodiversity, Ocean University of China, Yushan Road, No. 5, Qingdao, Shandong 266003, China

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ABSTRACT

After a *SUC2* gene in *Saccharomyces* sp. W0, isolated from fermented rice was removed, the disruptant W4 obtained could not produce any invertase, even could not grow in the media containing sucrose and inulin, but could grow in the medium containing fructose. At the same time, inulin was not hydrolyzed by the disruptant. However, after the *SUC2* gene was over-expressed in the disruptant W4, the recombinant yeast strain Suc2-1 obtained could produce much higher invertase activity than *Saccharomyces* sp. W0 and more inulin was hydrolyzed and the transcriptional level of the *SUC2* gene in the recombinant yeast strain Suc2-1 was also much higher than that in *Saccharomyces* sp. W0. This is the first time to report that the *SUC2* gene in *Saccharomyces* sp. W0 is closely related to both invertase activity and inulin hydrolysis. The invertase over-produced by the recombinant yeast strain Suc2-1 could actively convert inulin into monosaccharides. The recombinant yeast strain Suc2-1 over-expressing the *SUC2* gene could produce over 13.4% (v/v) ethanol from 300.0 g/L inulin directly, suggesting that *Saccharomyces* sp. W0 over-producing invertase also can be applied to ethanol fermentation industry.

1. Introduction

Inulin, an important carbohydrate polymer in the roots and tubers of plants such as Jerusalem artichoke, consists of linear chains of β-2,1-linked D-fructofuranose molecules terminated by a glucose residue through a sucrose-type linkage at the reducing end [1]. In recent years, microbial inulinases have received increasing attention because they can be used to hydrolyze inulin and inulin-containing materials such as extract of Jerusalem artichoke tubers for production of ethanol, biodiesel, single cell protein, citric acid, ultra-high-fructose syrup, inulooligosaccharides, 2,3-butanediol, lactic acid, sugar alcohols and so on [2]. Inulinases are fructofuranosyl hydrolases that target on the β-2,1 linkage of inulin and hydrolyze it into fructose and glucose. According to their action, inulinases are divided into exo-inulinase (E.C.3.8.1.80) which hydrolyzes terminal, non-reducing 2,1-linked and 2,6-linked β-D-fructofuranose residues in fructans, with concomitant release of β-D-fructose and glucose and endo-inulinase (E.C.3.2.1.7) that breaks down internal linkages in inulin molecules to yield inulooligosaccharides (IOS) including inulotriose, inulotetraose and inulopentaose, but lacks invertase activity [1]. Inulinases are the members of glycoside hydrolase family 32 (GH32) and the active site of the GH32 is characterized by the presence of at least six highly conserved motifs. In exo-inulinases, the sequences of amino acids of the conserved motifs 1, 2, 3, 4, 5 and 6 are WMNDPNGL, RDP, ECP, SVEVF, FS and Q, respectively whereas in endo-inulinase, the sequences of amino acids of the conserved motifs 1, 2, 3, 4, 5 and 6 are WMNEPNGL, RDP, EVP, SVEVF, FT and Q, respectively [3]. However, the conserved motif, SVEVF, does not appear in yeast exo-inulinase. It has been reported that the sequence plays an important role in binding of the high-Mr fructans [3].

Invertase (β-D-fructofuranoside fructohydrolase, E.C.3.2.1.26) is beta-fructofuranosidase that catalyzes hydrolysis of the terminal non-reducing beta-fructofuranoside residues in beta-fructofuranosides [4]. It also belongs to the member of glycoside hydrolase family 32 (GH32) and is widely present in bacteria, yeasts, fungi, higher plants and in some animal cells. Especially, invertase from *Saccharomyces cerevisiae* has been intensively investigated and applied to food and fermentation industries [5] and secreted invertase was mainly located in the periplasmic space of the yeast [6]. After analysis of the invertase in *S. cerevisiae* and inulinases in other microorganisms, it was found that like yeast exo-inulinase, the invertase from *S. cerevisiae* indeed contained such conserved sequences, WMNDPNGL, RDP, ECP, FS and Q, but not SVEVF (Fig. 1). Therefore, it is possible for the invertase to have exo-inulinase activity. In our previous study [7], it was found that *Saccharomyces* sp. W0 used in this study can ferment inulin and produce low concentration of ethanol from inulin directly. Wang and

* Corresponding author. Tel.: +86 532 82032266; fax: +86 53282032266.
E-mail address: zhenming@sdu.edu.cn (Z.-M. Chi).

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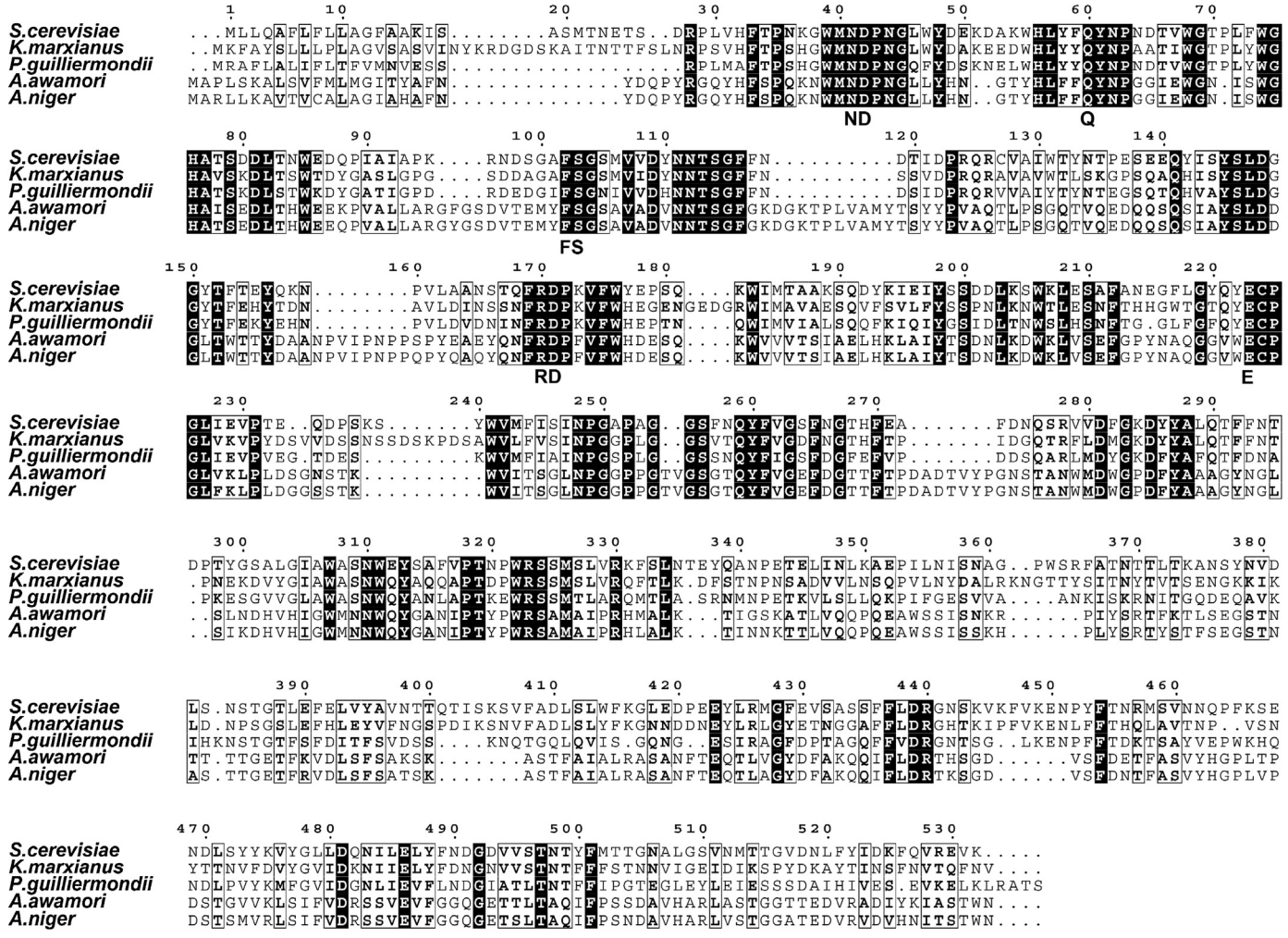

Fig. 1. Multiple alignment of the deduced amino acid sequences of invertase from *S. cerevisiae* and exo-inulinases from other microorganisms. The alignment was performed using CLUSTALW. *S. cerevisiae* (Invertase, accession number: NP_012104), *Kluyveromyces marxianus* (Inulinase, accession number: CAA40488), *Pichia guilliermondii* (Inulinase, accession number: ABW70125), *Aspergillus niger* (Inulinase, accession number: DQ233222), *Aspergillus ficuum* (Inulinase, accession number: ADM21204), *Aspergillus awamori* (Inulinase, accession number: CAC44220).

Li [8] reported that an invertase produced by *S. cerevisiae* had low inulinase activity and hydrolyzed inulin with low molecular weight. In fact, twenty years ago, it was found that an invertase from *S. cerevisiae* preferentially cleaved sucrose, but could also hydrolyze inulin at low efficiency [9]. However, little is known about relationship between the invertase gene (*SUC2*) and inulin hydrolysis and ethanol production from inulin in *S. cerevisiae.* It has been reported that there are six different genes, *SUC1–SUC5* and *SUC7* in *S. cerevisiae* that code for very similar electrophoretic variants of invertase [10] and among them, the *SUC2* gene encodes the main invertase activity. Therefore, in this study, the *SUC2* gene was disrupted in *Saccharomyces* sp. W0, a high ethanol producing yeast, and the native *SUC2* gene was over-expressed in the disruptant. Then, the relationship between the invertase gene and invertase activity, inulin hydrolysis and ethanol production was examined.

2. Materials and methods

2.1. Strains, plasmids and media

Escherichia coli DH5α [*supE44 DlacU169 (B80lacZDM15) hsdR1recA1 endA1 gyrA96 thi-1 relA1*] was used to amplify the plasmids carrying the cloned invertase gene and other DNA fragments. The *E. coli* transformants were grown in LB medium with 100.0 μg/mL of ampicillin. *Saccharomyces* sp. W0 which is a high ethanol-producing yeast isolated from the fermented rice and was able to ferment inulin into ethanol [11,12] was used as a control in this study. The uracil mutant W12d of *Saccharomyces* sp. W0 was obtained in the previous study and used for the *SUC2* gene disruption [13]. Yeast growth medium was the YPD medium containing 20.0 g/L glucose, 20.0 g/L peptone, 10.0 g/L yeast extract. The invertase production medium contained 20.0 g/L sucrose or 20.0 g/L inulin, 10.0 g/L yeast extract and 20.0 g/L polypeptone. The medium used for ethanol production from inulin was composed of 300.0 g/L inulin and 20.0 g/L malt extract. The homologous integration expression vector pMIDSC11 which carries a delta-sequence in *S. cerevisiae* and pDC10 carrying the promoter of the *TEF1* gene, the *HPT* gene and terminator *CYC1* were constructed by Wang et al. [14]. pMD19-T simple vector (TaKaRa, Japan) was used to transform *E. coli* DH5α.

2.2. Isolation of DNA, restriction digestions, and transformation

DNA manipulations were performed using standard methods [15]. Bacterial plasmid DNA was purified using Perfect-prep plasmid mini kits (Eppendorf). Yeast genomic DNA for amplification of the extracellular invertase gene in *Saccharomyces* sp. W0 was isolated as described by Chi et al. [16]. Restriction endonuclease