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



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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, 2,3butanediol, 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

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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.

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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 exoinulinase. 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) beta-fructofuranosidase that catalyzes hydrolysis of the is terminal non-reducing beta-fructofuranoside residues in betafructofuranosides [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

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S.cerevisiae K.marxianus P.guilliermondii A.awamori A.niger	1 10 MLLQAFLFLLAGFAAKIS MKFAYSLLEPLAGVSASVI MRAFLALIFLTFVMNVESS MAPLSKALSVFMLMGITYAFN MARLLKAVTVCALAGIAHAFN	NYKRDGDSKAITNTTFSI	LNRPSVHFTPSHGWMNDP RPLMAFTPSHGWMNDP PYRGOYHFSPOKNWMNDP	NGLWYDEKDAKWHLYE NGLWYDAKEEDWHLYY NGQFYDSKNELWHLYY NGLLYHN. GTYHLFE	QYNPAATIWGTPLYWG QYNPNDTVWGTPLYWG QYNPGGIEWGN.ISWG
S.cerevisiae K.marxianus P.guilliermondii A.awamori A.niger	80 90 HATSDDLTNWEDOFTATAPK. HAVSKDLTSWTDYCASLGPG. HATSKDLSTWEDYGATIGPD. HATSEDLTHWEEKPVALLARG HATSEDLTHWEEQPVALLARG	100 11 RNDSGAFSGSMVVD SDDAGAFSGSMVID RDEDGIFSGNIVVD FGSDVTEMYFSGSAVAD YGSDVTEMYFSGSAVAD FS	YNNTSGFFN	120 .DTIDPRORCVATWT .SSVDPRORAVAVWT .DSIDPRORVAUVT YTSYYPVAOTLPSGO YTSYYPVAOTLPSGO	140 NTPESEEOYISYSLDG SKGPSOAOHISYSLDG
S.cerevisiae K.marxianus P.guilliermondii A.awamori A.niger	150 160 GYTFTEYQKN	170 18	9 199 50KWIMTAAKSQDY ENGEDGRWIMAVAESQVF TNQWIMVIALSQQF 50KWVVVTSIAELH 50KWVVVTSIAELH	200 KIEIYS SDDTKSWKI SVLFYS SPNLKNWTL KIQIYGSIDLTNWSD KLAIYTSDNLKDWKL KLAIYTSDNLKDWKL	
S.cerevisiae K.marxianus P.guilliermondii A.awamori A.niger	230 GLIEVPTE. QDPSKS GLVKVPYDSVVDSSNSSDSKF GLIEVPVEG.TDES GLVKLPLDSGNSTK GLFKLPLDGGSTK	240 250	260 270	28	30 290
S.cerevisiae K.marxianus P.guilliermondii A.awamori A.niger	300 310 DPTYGSALGTAMAGNWEYSAF .PNEKDVYGIAWASNWOYAQQ .PKESGVVGLAMASNWOYANI SLNDHVHIGWMNNWOYGAN SIKDHVHIGWMNNWOYGAN	APTKEWRSSMTLARQMT IPTYPWRSAMAIPRHMA	A.SRNMNPE TK V LS LLQ	KPIFGE SV VAAN EAWSSI SN KR	IKISKRNITGQDEQAVK PIYSRTFKTLSEGSTN
S.cerevisiae K.marxianus P.guilliermondii A.awamori A.niger	390, 400, LS.NSTGTLEFELVYAVNTTC LD.NPSGSLEFHLEXVFNGSF IHKNSTGTFSFDITFSVDSS. TT.TTGETFKVDLSFSAKSK. AS.TTGETFRVDLSFSATSK.	TISKSVFADL <mark>SL</mark> WFKGLI DIKSNVFADL SL YFKG N I	NDDNEYLRLGYETNGGAF	FLDRGHTKIPFVKENI	FFTHOLAVTNP VSN
S.cerevisiae K.marxianus P.guilliermondii A.awamori A.niger	470, 480, 49 NDLSYYKVYGLLDONILELYF YTTNVFDVYGVIDKNIIELYF NDLPVYKMFGVIDGNLISVFL DSTGVVKLSIFVDRSSVEVFG DSTSMVRLSIFVDRSSVEVFG	NDGDVVSTNTYFMTTGN DNGNVVSTNTFFFSTNN NDGIATLTNTFFIPGTE GQGETTLTAQIFPSSDA	VIGEIDIKSPYDKAYTIN GLEYLEIESSSDAIHIV VHARLASTGGTTEDVRAD	SFÑVTQFNV S.EVKELKLRATS IYKIASTWN	

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 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 DH5 α [*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

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