Kor. J. Appl. Microbiol. Biotechnol.
Vol. 20, No. 6, 642-646 (1992)

Isolation and Characterization of *Saccharomyces cerevisiae* Mutants Deficient in (1→3)-β-D-Glucan Synthase

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베타-1,3-글루칸 생합성능이 손상된 *Saccharomyces cerevisiae* 돌연변이체의 선별 및 특성

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Abstract — We have isolated conditional lethal mutants of *Saccharomyces cerevisiae* which are low in (1→3)-β-D-glucan synthase activity. These mutants were osmotic sensitive at nonpermissive temperature (37℃) and showed a decreased level of alkali-insoluble cell wall glucan. The decrease in (1→3)-β-D-glucan synthase activity of the mutants appeared to be mainly due to the defect in catalytic component rather than in GTP-binding component.

Fungal cell wall is a potential target for antifungal agents because of its importance in growth and development (5). However, the architecture and synthesis of the fungal cell wall are poorly understood. In *Saccharomyces cerevisiae*, cell wall β-glucan, a homopolymer of glucose units linked through either (1→3)-β- or (1→6)-β-D-glycosidic bonds, is one of the major structural components which support and maintain the cell wall structure. Nothing is known about the biochemistry of (1→6)-β bond formation, but valuable information has been accumulated on the biosynthesis of (1→3)-β-D-glucan. The synthesis of (1→3)-β-D-glucan by a mixed membrane fraction from *S. cerevisiae*, with UDP-glucose as a glycosyl donor, was stimulated by nucleoside triphosphates and its analogs (4). The membrane preparation of (1→3)-β-D-glucan synthase was dissociated into two soluble components, GTP-binding component and presumed catalytic component, by differential extraction with detergents (6, 10). However, further study of (1→3)-β-D-glucan synthase system was hampered by the failure in purifying its constituents.

Because of the lack of mutants defective in the biosynthesis of cell wall (1→3)-β-D-glucan, little is known about the mechanism of (1→3)-β-D-glucan biosynthesis at the genetic and the molecular level. Although a mutant approach has been valuable in understanding the synthesis of other cell wall polysaccharides such as mannan (1) and chitin (3), this approach has been less informative for (1→3)-β-D-glucan synthesis. The searches for conditional lethals with a lysis phenotype or for resistance to drugs thought to inhibit (1→3)-β-D-glucan synthesis have not identified synthase mutants (13). To date only two classes of genes whose products are required for the synthesis of the major β-glucan of the yeast cell wall were reported. One class is *cwg*$^+$ genes of *Schizosaccharomyces pombe* (12) and the other is *KRE* genes of *S. cerevisiae* (13).

We present here the isolation and the partial characterization of conditional mutants of *S. cerevisiae*. They are osmotic sensitive and show a dec-

Key words: *Saccharomyces cerevisiae*, (1→3)-β-D-glucan synthase, lysis mutants, osmotic sensitivity
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rease in (1→3)-β-D-glucan synthase activity at non-permissive temperature, 37℃.

Materials and Methods

Saccharomyces cerevisiae GS-1-36 (α *SUC2 mal gal 2 CUP1,* ATCC 26180) provided by Cabib was mutagenized with ethyl methanesulfonate (15). Mutants that might be defective in cell wall synthesis were isolated by screening for the osmotically remediable phenotype in the presence of 1.2 M sorbitol at nonpermissive temperature, 37℃. To identify (1→3)-β-D-glucan synthase defective mutants, glucan synthase activity was measured with whole cell, digitonin-permeabilized cell, and membrane preparation from the cultures grown at 37℃.

The methods for the preparation of membrane fractions and the assay condition for the (1→3)-β-D-glucan synthase activity were the same as previously described (6, 8, 10). When the whole cell of mutants and digitonin-permeabilized cells were used as enzyme source, α-amylase (25 μg) was included in reaction mixture to prevent the incorporation of UDP-glucose into glycogen. Incorporation of radioactivity from UDP-[^{14}C]glucose into trichloroacetic acid insoluble material was determined according to Szaniszlo *et al.* (16). Protein was measured by the method of Lowry *et al.* (11) with bovine serum albumin as a standard.

Cell wall polysaccharide, total alkali-insoluble β-glucan, and (1→6)-β-glucan were prepared as outlined by Boone *et al.* (2). Total carbohydrate of each fraction was measured as hexose by phenol sulfuric acid method (7).

Results and Discussion

By ethyl methanesulfonate mutagenesis, 45 mutants showing osmotically remediable phenotype at nonpermissive temperature (37℃) were isolated. To identify (1→3)-β-D-glucan synthase defective mutants, we measured the activity of (1→3)-β-D-glucan synthase of 16 mutant strains grown at 37℃. As shown in Figure 1, most of the mutants showed lower level of (1→3)-β-D-glucan synthase activity. Interestingly, PS101, 210, and 211, showed the same

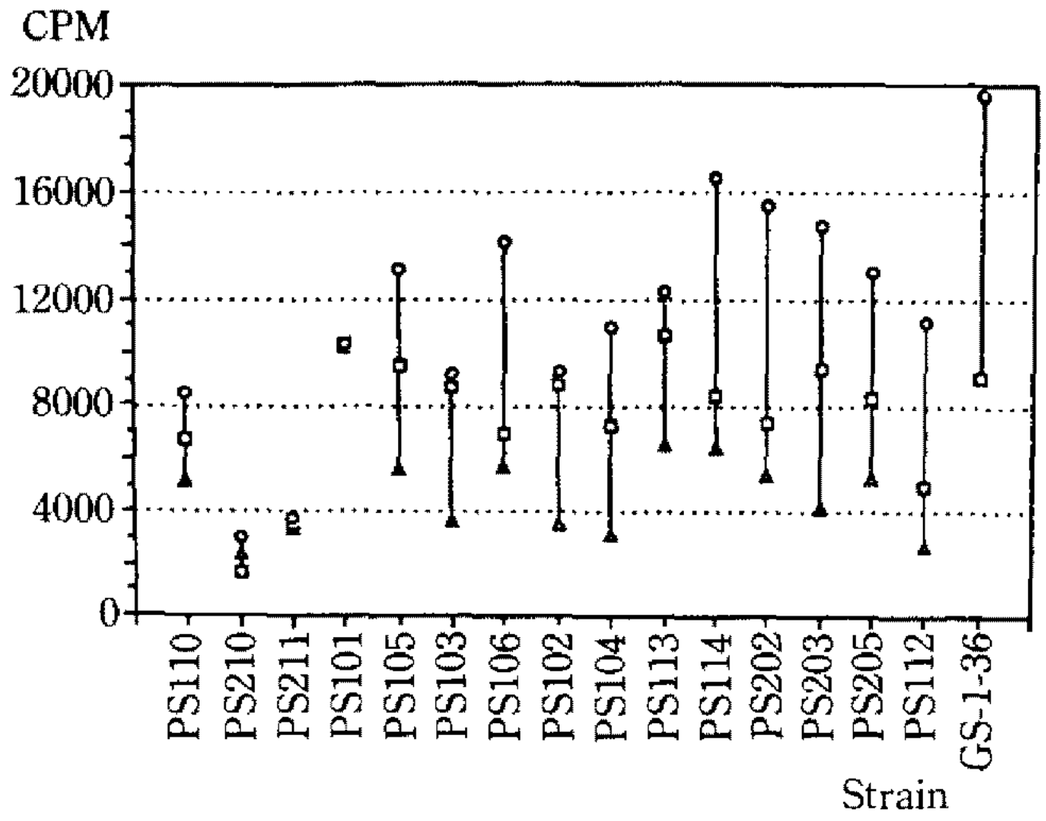

Fig. 1. (1→3)-β-D-glucan synthase activity of wild type and mutant strains.
Enzyme activity of each strain was assayed with whole cell (△), cell treated with 1% digitonin (□), and membrane preparation (○) from cultures grown at 37℃.

level of enzyme activity regardless of the enzyme sources used. The result indicates that these three mutants could have an increased cell permeability as the case of fragile mutants which had the phenotype of increased permeability for substances unable to penetrate wild type *S. cerevisiae* (17, 18).

There have been several reports on the isolation of conditional lysis mutants of *S. cerevisiae*; *cly* mutants by Hartwell (9), *mnn9* mutants by Ballou (1), and fragile mutant by Venkov *et al.* (17). None of these lysis mutants showed the decrease in (1→3)-β-D-glucan synthase activity. The only mutants of *S. cerevisiae* defective in (1→3)-β-D-glucan synthase activity were null mutants of *KRE6* obtained from one of the killer toxin resistant mutants by one-step gene disruption. These mutants showed reduced level of alkali-insoluble (1→3)- and (1→6)-β-D-glucan. Despite of the lack in a significant amount of wall β-glucan, these mutants were still viable and not osmotically sensitive (13). As can be seen in Table 1, PS110, 210, and 211 showed 61 to 86.5% reduction of *in vitro* enzyme activity and conditional lethality at the nonpermissive temperature. It colud be concluded, therefore, that the mutants reported here are a novel class of *S. cerevisiae* lysis mutants, which have similiar characteristics of the thermosensitive mutants of *S. pombe* requring the presence of an osmotic stabilizer to survive and grow at non-

permissive temperature and showing defect in the (1→3)-β-D-glucan synthase (12). The morphologies of the three mutants, PS110, PS210, and PS211, grown at 37℃ in the presence of 1.2 M sorbitol are shown in Figure 2.

In order to ascertain that the content of (1→3)-β-D-glucan was affected by the mutation, the level of alkali-insoluble β-glucan was measured. These mutants showed a decrease in the level of alkali-insoluble β-glucan even at the permissive temperature (Table 2). Although all the three mutant strains showed reduction in (1→3)-β-D-glucan synthase activty as well as (1→3)-β-D-glucan content, there was not a clear correlation between them. Most interestingly, PS210, which showed normal growth and cell wall (1→3)-β-D-glucan content, showed decreased *in vitro* activity of (1→3)-β-D-glucan synthase even at the permissive temperature, 25℃. We cannot rule out the possibility that more than two enzymes could participate in the biosynthesis of cell wall (1→3)-β-D-glucan in *S. cerevisiae* as the case of chitin synthase (14) and of (1→6)-β-D-glucan biosynthesis (13). Definitive evidence will come from the genetic analysis and cloning of the genes. We do not know yet whether the osmotic sensitivity of mutants at nonpermissive temperature was caused by the defect in (1→3)-β-D-glucan synthase activity and/or in other gene products which confer the osmotic stability of yeast cell.

Table 1. Effect of temperature on the growth and (1→3)-β-glucan synthase activity

Strain	Temperature (℃)	Growth	(1→3)-β-glucan synthase activity (mol/h·mg protein)	%[a]
GS-1-36	25	+	228.9	100.0
	30	+	296.2	100.0
	37	+	381.0	100.0
PS110	25	+	ND[b]	
	30	+	163.7	55.3
	37	−	148.6	39.0
PS210	25	+	107.0	46.8
	30	+	98.8	33.4
	37	−	51.4	13.5
PS211	25	+	104.5	45.7
	30	+	88.4	29.8
	37	−	65.4	17.2

[a] The enzyme activity of GS-1-36 at a given temperature was taken as 100%.
[b] Not determined.

Table 2. Levels of alkali-insoluble β-glucans in wild type and mutants

Strain	Temperature (℃)	β-glucan, μg/mg cell wall dry wt		
		Total[a]	(1→6)	(1→3)[b]
GS-1-36	25	718.3	127.0	591.3 (100)[c]
	37	864.7	130.0	734.7 (100)
PS110	25	345.0	88.0	257.0 (43.5)
	37	180.0	28.1	151.9 (20.7)
PS210	25	668.3	76.1	592.2 (100)
	37	485.9	59.3	426.6 (58.1)
PS211	25	505.4	60.3	445.1 (75.3)
	37	166.5	57.0	109.5 (14.9)

[a] The amount was determined as the sum of the carbohydrate content of both the Zymolyase-insoluble pellet and the solubilized supernatant before dialysis.
[b] The amount was determined as the difference of the carbohydrate content between total and (1→6)-β-glucan.
[c] The amount of (1→3)-β-glucan of GS-1-36 at a given temperature was taken as 100.

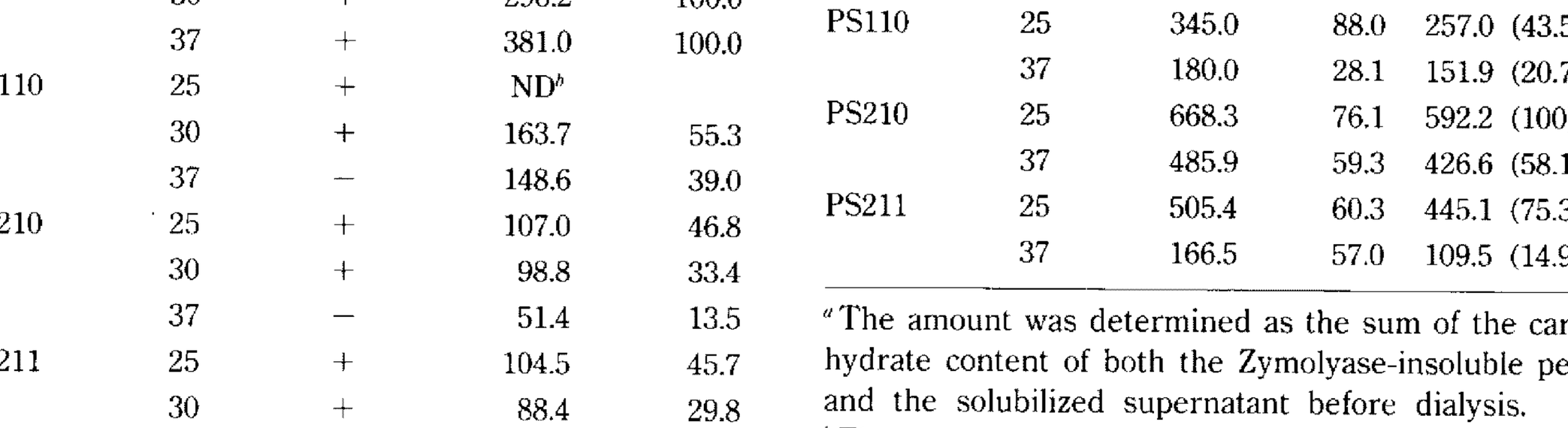

Fig. 2. Morphology of wild type and mutant strains viewed by light microscope.
Cells were grown at 37℃ in the presence of 1.2M sorbitol. a, GS-1-36; b, PS110; c, PS210; d, PS211 (×400).

Table 3. Effect of GTP-binding component on particulate catalytic component from wild type and mutant strains grown at 37°C

Origin of GTP-binding component	Glucan synthase activity, cpm				
	No catalytic component	GS-1-36	PS110	PS210	PS211
No GTP-binding component added	–	1482	1419	1429	1694
GS-1-36	1688	10724	2651	2479	2042
PS110	1557	9304	3343	2264	2401
PS210	2010	6312	1853	1640	2129
PS211	1795	6923	2346	2883	2277

Reconstitution experiments were performed to determine which component was affected by mutations. As shown in Table 3, when each of the particulate catalytic component from (1→3)-β-D-glucan synthase defective mutants was reconstituted with the solubilized GTP-binding component from wild type strain, no stimulation of enzyme activity was observed. On the contrary, when each of the GTP-binding component from PS110, PS210 and PS211 was added to the catalytic component from wild type, the (1→3)-β-D-glucan synthase activity was 86.6, 58.8, and 64.6% of wild type strain, respectively. It seemed more probable that the reduction of enzyme activity in the (1→3)-β-D-glucan synthase defective mutants might be mainly due to the defect in catalytic component as the case of *cwg1-1* mutant of *S. pombe* (12). These results, however, would not exclude the possiblity of partial defect in GTP-binding component or in other factors involved in glucan synthase system.

In this communication we present that the glucan synthase defective mutants of *S. cerevisiae* were isolated from the lysis mutants. The mutants obtained in this study are a novel kind of lysis mutants of *S. cerevisiae* that have distinct properties such as the deficiency in (1→3)-β-D-glucan synthase and the conditional osmotic sensitivity. These characteristics are different from those of any other lysis mutants of *S. cerevisiae* reported previously. These mutants would make us enable to perform the genetic and molecular study on the gene(s) involved in the cell wall glucan biosynthesis of fungi.

요 약

본인 등은 베타-1,3-글루칸 합성효소능이 낮은 *Saccharomyces cerevisiae*의 조건성 돌연변이주들을 분리하여 그 특성을 조사하였다. 이 돌연변이주들은 비허용온도인 37℃ 에서 삼투감수성을 나타나며, 세포벽 성분 중 알칼리 비용해성 글루칸의 함량이 낮았다. 베타-1,3-글루칸 합성효소능의 결함원인을 조사한 결과, 효모의 베타-1,3-글루칸 합성효소를 구성하는 촉매성분(catalytic component)과 GTP-결합성분 두 가지 중 촉매성분의 결합이 이 돌연변이주들의 베타-1,3-글루칸 합성효소능의 손상원인인 것으로 추정되었다.

Acknowledgements

We thank to E. Cabib for useful criticism. We also thank to P.J. Maeng and J. Kim for correcting the manuscript. This work was supported by Research Fund for Promotion of Local University (1990) from Ministry of Education, Korea.

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(Received November 24, 1992)