

## Screening System for Chitin Synthase II Inhibitors from Natural Resources and its Inhibitor Prodigiosin

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**Abstract** Chitin synthases are identified as key enzymes of chitin biosynthesis in most of the fungi. Among them, chitin synthase II has been reported to be an essential enzyme in chitin biosynthesis, and exists as a membrane-bound form. To search and screen new antifungal agents from natural resources to inhibit chitin synthase II, the assay conditions were established using the enzyme isolated from *Saccharomyces cerevisiae* ECY38-38A(pAS6) that overproduces only chitin synthase II. This enzyme was activated only by partial proteolysis with trypsin. Its activity reached the maximum at 80 µg/ml of trypsin and was strongly stimulated by 2.0 mM Co<sup>2+</sup>, 1.0 mM UDP-[<sup>14</sup>C]-GlcNAc, and 32 mM free-GlcNAc. Under these assay conditions, the highest chitin synthase II activity was observed by incubation at 30°C for 90 min. However, an extremely narrow range of organic solvents up to as much as 25% of DMSO and 25% of MeOH was useful for determining optimal assay conditions. After a search for potent inhibitors of chitin synthase II from natural resources, prodigiosin was isolated from *Serratia marcescens* and purified by solvent extraction and silica gel column chromatographies. The structure of prodigiosin was determined by UV, IR, Mass spectral, and NMR spectral analyses. Its molecular weight and formula were found to be 323 and C<sub>20</sub>H<sub>25</sub>N<sub>3</sub>O, respectively. Prodigiosin inhibited chitin synthase II by 50% at the concentration of 115 µg/ml.

**Key words:** Chitin synthesis, chitin synthase II inhibitor, antifungal agents, prodigiosin

During the last three decades, there has been a dramatic increase in the frequency of fungal infections, especially that of disseminated systemic mycoses in the immunodeficient host [14]. Mycoses in immunocompromised hosts are mainly the result of opportunistic infections by organisms

that are normally harmless, asymptomatic commensals which can be, under certain conditions, pathogenic [27]. Species of *Aspergillus*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, and *Sporothrix* are all known for their important causative agents. Presently, treatments for fungal infections are limited to only a few therapeutic options [28]. Current drugs include amphotericin B and a variety of azoles. Unfortunately, amphotericin B is identified to be toxic to humans, and clinical resistance to azoles is rising [21, 25].

Chitin, the β-1,4-linked homopolymer of *N*-acetyl-D-glucosamine (GlcNAc), is an important structural component of the cell wall of mostly zoopathogenic and phytopathogenic fungi [5, 15]. Because chitin is not found in mammals and plants, specific inhibitors of chitin synthesis must show fungal selectivity together with desirable toxicological and environmental properties, which should be under consideration for the development of antifungal agents [1]. In *Saccharomyces cerevisiae*, chitin is synthesized by chitin synthase I, II, and III, and their functional and enzymatic characteristics are quite different [2-5, 7, 10, 11, 19, 26, 29-32]. Chitin synthase II is an essential enzyme for primary septum formation and cell division, whereas chitin synthase III is responsible for chitin ring formation at bud emergence and in the lateral cell wall and for the formation of glucan-chitin linkage. However, chitin synthase I is a nonessential enzyme that repairs the cell wall. Three chitin synthases have also been found in *Candida albicans*. In this organism, chitin synthase I, an equivalent of chitin synthase II from *S. cerevisiae*, is involved in septum formation [9, 21, 24]. Although three chitin synthases from both *S. cerevisiae* and *C. albicans* are located primarily in the plasma membrane and they are present in cell homogenates largely as zymogens, they exhibit specific enzymatic properties in terms of their requirements for certain factors.

In the present study, we investigated factors affecting chitin synthase II activity isolated from *S. cerevisiae*

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ECY38-38A(pAS6) in a search and development of novel chitin synthase II inhibitors from natural resources. Using this assay system, we have isolated 18 samples from microorganisms and plants with inhibitory activities against chitin synthase II, and the isolation, structure determination, and biological activity of prodigiosin, which was derived from *S. marcescens*, is reported in this paper.

## MATERIALS AND METHODS

### Yeast Strain and Culture Conditions

The strain used in this study was *Saccharomyces cerevisiae* ECY38-38A(MATa *chs1-23 chs2::LEU2 cal1/csd2 ura3-52 trp1-1 leu2-2* pAS6) grown in YPG (1% yeast extract, 2% peptone, 2% galactose), which can overexpress only the chitin synthase II.

### Membrane Preparation

Membrane was prepared as described by Choi and Cabib [11]. Briefly, cells were suspended in 50 mM Tris-HCl (pH 7.5) containing 5 mM magnesium acetate, and were broken by vortex mixing with glass beads (Sigma, 425–600 microns). Cell walls were sedimented at 4,000 ×g for 5 min and the resulting supernatant was centrifuged at 130,000 ×g for 45 min. The membrane fraction was resuspended in 50 mM Tris-HCl (pH 7.5) containing 33% glycerol, to a final volume of 1.6 ml/g (wet weight) of cells used.

### Chitin Synthase II and III Assay

Chitin synthase activity was measured by the procedure previously described [11]. To establish optimal enzymatic assay conditions, we investigated the effects of various concentrations of factors such as trypsin, divalent cations, substrate, incubation time, and solvents which affected chitin synthase II activity. For proteolytic activation of chitin synthase II, the reaction mixtures contained 32 mM Tris-HCl (pH 8.0), 2.0 mM cobalt acetate, 1.0 mM UDP-[<sup>14</sup>C]-GlcNAc (400,000 cpm/μmol, NEN), 2 μl of trypsin solution (2.0 mg/ml) (Sigma, T-8642), 20 μl of membrane preparation, and 14 μl of sample in a total volume of 46 μl. The mixtures were incubated for 15 min at 30°C. Proteolysis was stopped by adding 2 μl of a soybean trypsin inhibitor solution (4.0 mg/ml) at a concentration twice that of the trypsin used, and the tubes were placed on ice for 10 min. GlcNAc was added to a final concentration of 32 mM and it was further incubated at 30°C for 90 min. The insoluble chitin formed after adding 10% of trichloroacetic acid and filtration through a glass fiber filter (GF/C, Whatman) was assayed by measuring radioactivity. For chitin synthase III activity, the membrane was prepared from *Saccharomyces cerevisiae* ECY38-38A(MATa *chs1-23 chs2::LEU2 cal1/csd2 ura3-52 trp1-1 leu2-2* pWJC6),

which can overexpress only the chitin synthase III under the control of the *GALI* promoter [10], and the assay was performed by the same method as chitin synthase II except that 32 mM Tris-HCl (pH 7.5) and 4.3 mM magnesium acetate were used [11]. The concentration of protein was measured by the method described by Lowry [22]. Blank values were measured with the addition of 25% aqueous MeOH instead of enzyme and sample. Percent inhibition of chitin synthase II activity was calculated by subtracting blank values from both control and test sample values.

$$\% \text{ Inhibition} = \left[ 1 - \frac{\text{Sample (cpm)} - \text{Blank (cpm)}}{\text{Control (cpm)} - \text{Blank (cpm)}} \right] \times 100$$

The chitin synthase II and III activities were confirmed by positive control with polyoxin D and nikkomycin Z (Calbiochem Co.). Specific activity is expressed as the nanomoles of GlcNAc incorporated per hour per milligram protein.

### Preparation of Extracts from Natural Resources

Microorganisms isolated from soil in Korea were incubated at 25–30°C for 4–5 days in a medium of actinomycetes (2% starch, 0.4% soytone, 0.3% pharmamedia, 0.2% polypeptone, 0.1% yeast extract, 0.1% K<sub>2</sub>HPO<sub>4</sub>, 0.05% MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.2% NaCl, 0.3% CaCO<sub>3</sub>, 0.002% FeSO<sub>4</sub>, 0.001% MnCl<sub>2</sub>, 0.001% ZnSO<sub>4</sub>, and 0.0005% CoCl<sub>2</sub>) or in a medium of fungi (2% starch, 0.4% soytone, 0.3% pharmamedia, 0.1% K<sub>2</sub>HPO<sub>4</sub>, 0.05% MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.5% NaCl, 0.3% CaCO<sub>3</sub>, 20 mg FeSO<sub>4</sub>, 10 mg MnCl<sub>2</sub>, 0.5 mg ZnSO<sub>4</sub>, and 5 mg CoCl<sub>2</sub> in 1 l distilled water). After the cultivation, the fermentation broth was extracted with an equal volume of ethyl acetate by shaking and the extract was concentrated *in vacuo* to dryness. Plants were also extracted with methanol and then kept at room temperature for 5 days, and the mixtures were filtered through filter papers (Whatman No. 2) and the solvent was evaporated to dryness. All dried extracts were dissolved in 25% MeOH or 25% DMSO and were used for assay.

### Fermentation, Isolation, and Purification of Chitin Synthase II Inhibitor

A slant culture of *S. marcescens* grown in LB agar was used to inoculate to a 60-ml test tube containing 10 ml LB medium (Difco Co.), and the tube was shaken on a reciprocal shaker for 2 days at 26°C. One-ml of the seed culture was inoculated into a 500-ml baffled flask containing 50 ml LB medium and shaken on a reciprocal shaker for 2 days at 26°C. A 30 ml of culture was transferred into a 5-l baffled flask containing 1 l of production medium (1% starch, 0.5% pharmamedia, 0.2% glucose, 0.1% (NH<sub>2</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% K<sub>2</sub>HPO<sub>4</sub>, 0.05% MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.1% CaCl<sub>2</sub>, 0.3% NaCl, 0.0001% FeSO<sub>4</sub> · 7H<sub>2</sub>O, 0.0001% MnSO<sub>4</sub> · 7H<sub>2</sub>O, 0.1% L-alanine, 0.1% L-histidine, 0.1% L-proline, pH 7.0). The fermentation was carried out

for 48 h at 26°C on a rotary shaker at 150 rpm. The fermentation broth was filtered with diatomaceous earth. The filtrate was extracted with ethyl acetate (2×1 l) and the cell debris were washed twice with acetone (2×1 l). The ethyl acetate and acetone eluates were concentrated *in vacuo* to yield a red residue (1.29 g). It was dissolved in chloroform (1 l), filtered, and chromatographed on a silica gel (Merck, kieselgel 60, 230–400 mesh) column eluting with chloroform-methanol (98:2, v/v, 500 ml) to give a purified red compound (224 mg). The purified compound was characterized by EI-MS (Hewlett Parkard 5989A) and nuclear magnetic resonance spectrometry (NMR) (Varian UNITY 500).

## RESULTS

### Effect of Trypsin on Chitin Synthase II Activity

The chitin synthase II from various fungi exists in a zymogenic form and is converted into an active form by proteolytic action via protease treatment [5, 10, 11, 26, 29]. The chitin synthase II activity prepared from this strain was compared with or without trypsin treatment (Fig. 1). As expected, trypsin treatment of the enzyme increased the chitin synthase II activity. The optimal activity was achieved with 80 µg/ml of trypsin, which represented a 3.7-fold increase over the control.

### Specificity of Co<sup>2+</sup> and Mg<sup>2+</sup> on Chitin Synthase II Activity

Most of the enzyme preparations show a requirement for divalent cations, especially Mg<sup>2+</sup> (chitin synthase I, III) and Co<sup>2+</sup> (chitin synthase II) [5, 10, 11, 26, 29]. We investigated the effects of Co<sup>2+</sup> and Mg<sup>2+</sup> as stimulatory cations for chitin synthase II. As shown in Fig. 2, Co<sup>2+</sup> was the best stimulator of chitin synthase II from this strain (Fig. 2). The maximal activity was obtained by adding 2.0 mM

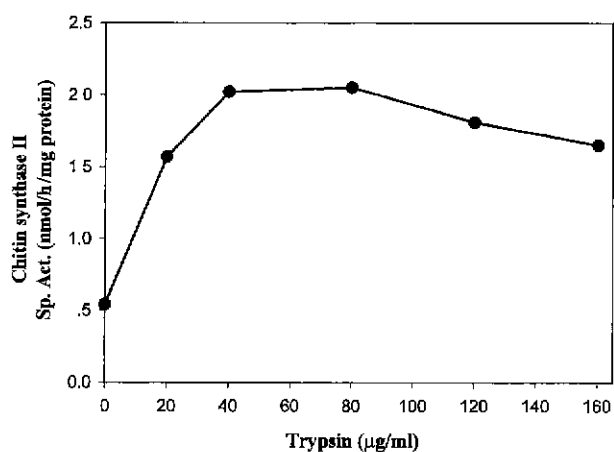


Fig. 1. Effect of trypsin on the activity of chitin synthase II.

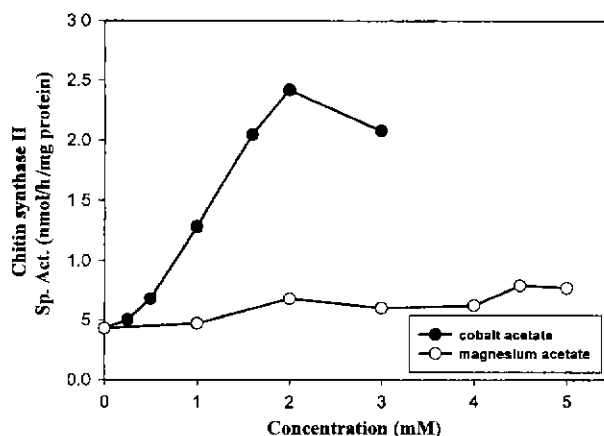


Fig. 2. Effects of Co<sup>2+</sup> and Mg<sup>2+</sup> on chitin synthase II activity. Both Co<sup>2+</sup> and Mg<sup>2+</sup> were added in their acetate form.

Co<sup>2+</sup>, which caused a 5.6-fold increase in the enzyme activity. Upon treatment with up to 5.0 mM Mg<sup>2+</sup>, the enzyme activity increased only slightly, indicating Co<sup>2+</sup> to be a definitely better stimulator than Mg<sup>2+</sup> for chitin synthase II.

### Effect of UDP-GlcNAc on Chitin Synthase II Activity

Because chitin is synthesized by incorporation of GlcNAc units from UDP-GlcNAc in the reaction catalyzed by chitin synthases, the chitin synthase II may be allosterically activated by its substrates, UDP-GlcNAc and GlcNAc [26, 27]. Therefore, we examined the effect of UDP-GlcNAc on chitin synthase II activity (Fig. 3). The optimal activity was obtained by 1.0 mM UDP-GlcNAc, which produced about a 1.5-fold increase, and the enzyme was saturated with substrate over this concentration. Although saturation was not clearly observed, the addition of 32 mM GlcNAc in the reaction mixture stimulated the enzyme activity by only about 1.5-fold (data not shown).

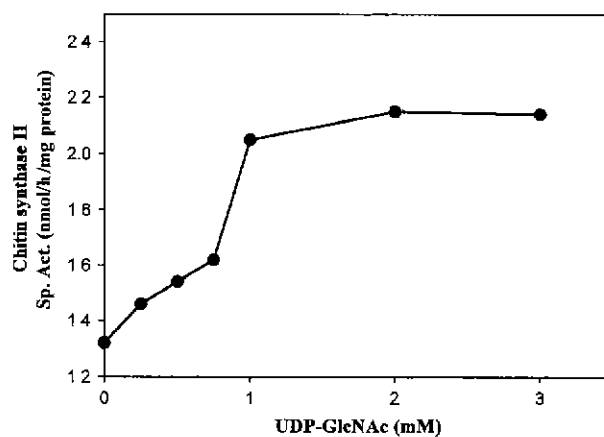


Fig. 3. Effect of UDP-GlcNAc on chitin synthase II activity. The prepared enzyme was treated with trypsin in the presence of the Co<sup>2+</sup> for 15 min at 30°C.

**Effects of Enzyme Concentration and Incubation Time on Chitin Synthase II Activity**

Because the amount of enzyme and incubation time are important parameters that affect enzyme activity *in vitro*, we have examined their effects on chitin synthase II activity. In a previous report, the maximum activity of chitin synthase II was obtained when the reaction mixture containing 6.4 mg/ml of the enzyme was incubated for 90–120 min at 30°C [11, 29]. According to our results, the activity of chitin synthase II was dependent on the enzyme concentration of up to 5.25 mg/ml and was saturated with substrate over this concentration (Fig. 4a). The highest chitin synthase II activity was observed when the reaction mixture was incubated with the enzyme for 90 min (Fig. 4b). This result corresponded well to that of Choi and Cabib [11] in which they used another recombinant, *S. cerevisiae* ECY36-3D(MATa *chs1-23 call1 ura3-52 leu2-2 trp1-1*), as the enzyme source.

**Effects of Solvents on Chitin Synthase II Activity**

Since the activity of chitin synthase II was dramatically inhibited in the various solvents, selecting an appropriate solvent system was quite a difficult task.

Under the assay conditions used, 25% of DMSO was selected to be the best solvent for the enzyme activity (Fig. 5) and 25% of MeOH, on the other hand, was slightly

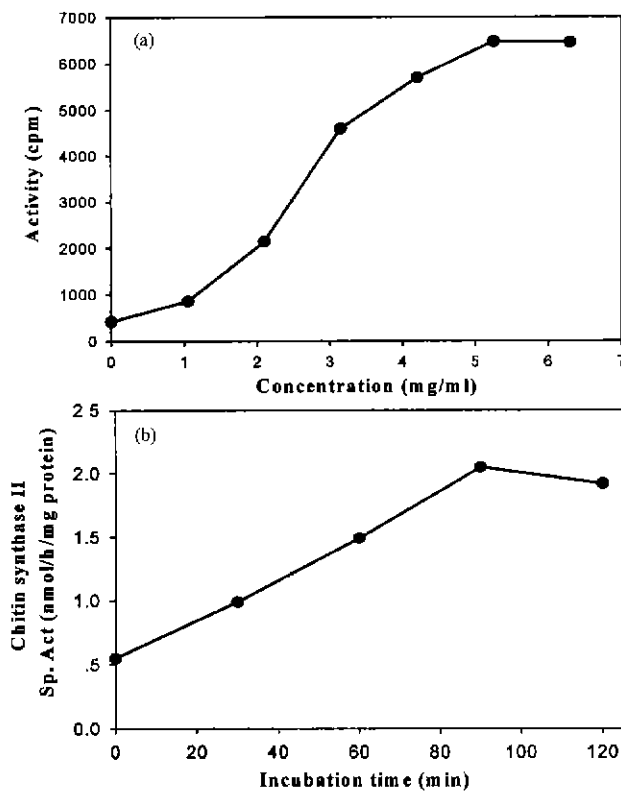


Fig. 4. Effects of crude enzyme (a) and incubation times (b) on the activity of chitin synthase II.

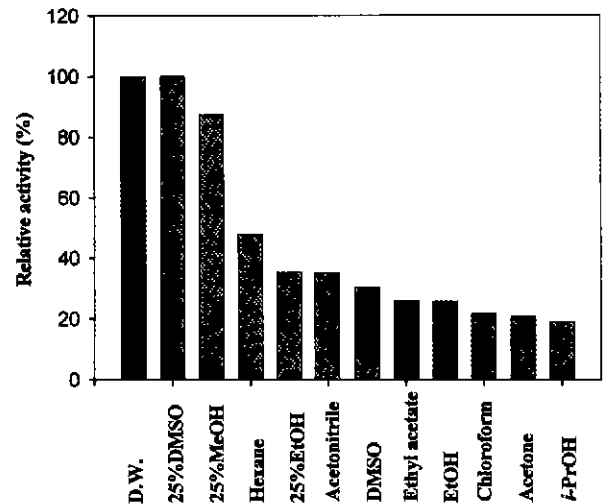


Fig. 5. Effects of solvents on the activity of chitin synthase II.

inhibitory. As compared with distilled water, inhibition (%) was almost zero in 25% of DMSO and 13% in 25% MeOH, respectively, but the other solvents such as hexane, ethyl acetate, acetone, ethanol, chloroform, acetonitrile, and *i*-PrOH were strongly inhibitory to the enzyme activity.

**Effects of Polyoxin D and Nikkomycin Z on Chitin Synthase II Activity**

The polyoxin D and nikkomycin Z are nucleoside tri- and di-peptide antibiotics, respectively, and their potent inhibitory activities against chitin synthase have been extensively studied [6]. They are analogues of UDP-GlcNAc and thus are competitive inhibitors of chitin synthases.

Under our assay conditions, both polyoxin D and nikkomycin Z effectively inhibited the enzyme activity. As shown in Fig. 6, the inhibition by polyoxin D and nikkomycin Z showed a dose-dependency on concentration up to

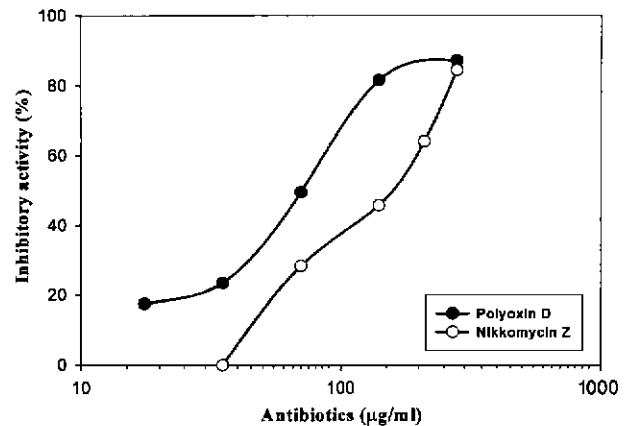


Fig. 6. Effects of polyoxin D and nikkomycin Z on the activity of chitin synthase II. Both polyoxin D and nikkomycin Z were dissolved in distilled water.

280  $\mu\text{g/ml}$  with  $\text{IC}_{50}$  of 70  $\mu\text{g/ml}$  and 176  $\mu\text{g/ml}$ , respectively. That is, polyoxin D was more effective than nikkomycin Z for the inhibition of chitin synthase II activity. This result confirms the earlier report that polyoxin D is a powerful and specific competitive inhibitor against chitin synthase II [6].

### Screening and Isolation of Chitin Synthase II Inhibitor from Natural Sources

In order to search and develop chitin synthase II inhibitors from natural sources, we have screened about 2,700 samples of microorganisms and plants in our country using chitin synthase II of *S. cerevisiae* ECY38-38A(pAS6) (Table 1). Among them, we isolated three candidates from microorganisms and fifteen candidates from plants, which showed more than 70% inhibition against chitin synthase II at the concentration of 280  $\mu\text{g/ml}$ . A secondary metabolite of microorganisms that showed strong inhibitory activity

**Table 1.** Screening of chitin synthase II inhibitors from natural resources.

Divisions	Samples	Candidates*
Microorganisms	2.601	3
Plants	100	15

\*The candidates showed more than 70% inhibitory activities at the concentration of 280  $\mu\text{g/ml}$ .

**Table 2.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data of prodigiosin from *S. marcescens*.

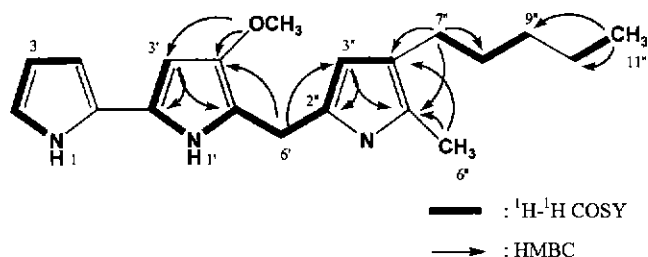
No	$^1\text{H}$ -NMR( $_{\text{H}}$ , J=Hz)	$^{13}\text{C}$ -NMR( $_{\text{C}}$ , ppm)
1	12.57 (1H, brs)	
2	7.21 (1H, m)	126.94
3	6.34 (1H, m)	111.71
4	6.90 (1H, m)	116.99
5		122.25
1'	12.71 (1H, brs)	
2'		147.69
3'	6.06 (1H, d, 2.0)	92.80
4'		165.75
5'		120.68
6'	6.94 (1H, brs)	116.01
2''		125.13
3''	6.66 (1H, brd, 2.4)	128.39
4''		128.49
5''		147.01
6''	2.52 (3H, s)	12.45
7''	2.37 (2H, t, 7.6)	25.31
8''	1.52 (2H, m)	29.79
9''	1.30 (2H, m)	31.41
10''	1.30 (2H, m)	22.49
11''	0.88 (3H, t, 7.1)	14.04
OCH <sub>3</sub>	3.99 (3H, s)	58.70

Measured in  $\text{CDCl}_3$ . S: singlet; d: doublet; t: triplet; m: multiplet; brs: broad singlet; brd: broad doublet.

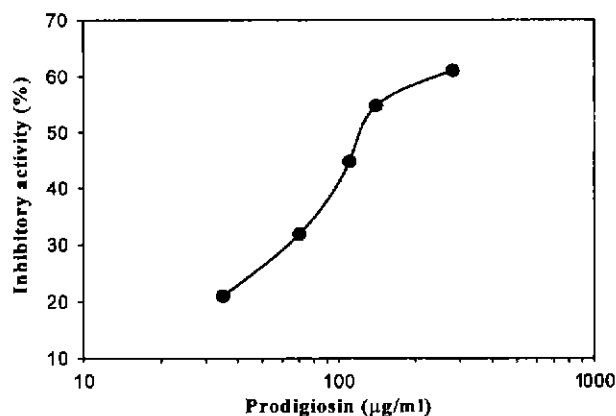
against chitin synthase II was isolated from the *S. marcescens* culture filtrate through several purification steps as described in Materials and Methods.

### Structure Determination and Biological Activity of Chitin Synthase II Inhibitor

Based on the EI-mass spectrum in combination with  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data, the molecular weight and formula of the isolated compound were found to be 323 and  $\text{C}_{20}\text{H}_{25}\text{N}_3\text{O}$ , respectively (Table 2). The structural determination of the isolated compound was carried out by NMR spectroscopic analyses including COSY, HMBC, and NOE (Fig. 7). This compound was proved to be prodigiosin by comparison of the spectral data with published data [12, 13]. Prodigiosin inhibited chitin synthase II in a dose-dependent manner up to 280  $\mu\text{g/ml}$ . The  $\text{IC}_{50}$  value of prodigiosin for chitin synthase II was 115  $\mu\text{g/ml}$ , representing 1.6 times-weaker and 1.6 times-stronger inhibitory activity than that of the previously identified chitin synthase inhibitor, polyoxin D ( $\text{IC}_{50}$ : 70  $\mu\text{g/ml}$ ) and nikkomycin Z ( $\text{IC}_{50}$ : 176  $\mu\text{g/ml}$ ), respectively (Fig. 8). This compound did not exhibit inhibitory activity against chitin synthase III from *S. cerevisiae* ECY38-38A(pWJC6) at the concentration of 280  $\mu\text{g/ml}$ . Prodigiosin exhibited very weak antifungal activity against pathogenic fungi (MIC: >400  $\mu\text{g/ml}$ ) such



**Fig. 7.**  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC experiments of the isolated compound ( $\text{CDCl}_3$ ). Arrows are directing H to C.



**Fig. 8.** Effect of prodigiosin on the activity of chitin synthase II. Prodigiosin was dissolved in 25% DMSO.

as *Candida albicans*, *C. krusei*, *Coccidioides immitis*, *Colletotrichum lagenarium*, *Fusarium oxysporum*, and *Pyricularia oryzae* (data not shown).

## DISCUSSION

Chitin has been widely known to be a selective target for antifungal agents because it was not found in mammalian cells. The chitin synthase of most fungi catalyzes the same biochemical reaction, using UDP-GlcNAc as a substrate to synthesize chitin. In *S. cerevisiae*, chitin is synthesized by chitin synthases I, II, and III, and their functional and enzymatic characteristics are quite different. However, the determination of individual activities from wild-type strain is difficult when all chitin synthases are present [5, 7, 10]. Since each enzyme is differentially affected by several factors such as pH, types of protease, and divalent cations [5, 10, 11], it is possible to examine optimal conditions for the measurement of each one of the three chitin synthases in the presence of the others.

In this study, optimal conditions are presented for assaying the chitin synthase II activity in a genetically engineered yeast strain, *S. cerevisiae* ECY38-38A(pAS6), that overproduces only chitin synthase II. Previously, Choi and Cabib [11], Orlean [26], and Sburlati and Cabib [29] found that the chitin synthase II from the yeast containing a disrupted *CHS1* gene was strongly activated with trypsin treatment and the maximal activity was obtained in the presence of  $\text{Co}^{2+}$ . The enzyme prepared from *S. cerevisiae* ECY38-38A(pAS6) was also activated by proteolysis with trypsin and it was strongly stimulated by  $\text{Co}^{2+}$ , indicating that this enzyme is a zymogen with traditional chitin synthase II properties. The chitin synthase II activity was significantly affected by the amount of enzyme, solvents used, and duration of incubation times. Sburlati and Cabib [29] reported that optimal conditions of chitin synthase II from *S. cerevisiae* D3B(*his chs1::URA3*) were 6.4 mg/ml enzyme at 30°C for 2 h, whereas we obtained slightly different results where the optimal enzyme concentration and incubation time were 5.25 mg/ml and 90 min, respectively. We suggest the difference was due to either the process of enzyme isolation or the strain selected.

In the course of screening chitin synthase II inhibitors from natural sources using our screening system, we found prodigiosin from *S. marcescens*. Prodigiosin, a characteristic red and water-insoluble pigment from *S. marcescens* also selectively inhibited chitin synthase II but not chitin synthase III activity. Prodigiosin has been reported to have antimalarial activity [8] and immunosuppressive activity [13], however, this is the first report for prodigiosin as a chitin synthase II inhibitor. Although prodigiosin is not

potent against chitin synthase II activity, this compound may serve as a useful lead compound for development of antifungal agents through the control of chitin biosynthesis. In addition, we have recently reported that a new group of chitin synthase II inhibitors, viz., ursolic acid [18], catechin [20], gomisins N [16], and chaetoatrosin A [17] were detected in *Crataegus pinnatifida*, *Taxus cuspidata*, *Shizandra chinensis*, and *Chaetomium atrobrunneum*, respectively.

According to these results, we are confident that this enzymatic assay system could be used as a screening system for antifungal agents in natural sources: the choice of enzymatic assay conditions is important when chitin synthesizing enzymes are used in screening potential antifungal agents, since each enzyme from each strain may have certain distinct enzymatic properties.

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## REFERENCES

1. Bartnki-Garcia, S. 1968. Cell wall chemistry, morphogenesis, and taxonomy of fungi. *Annu. Rev. Microbiol.* **22**: 87–102.
2. Bulawa, C. E. 1992. *CSD2*, *CSD3*, and *CSD4*, genes required for chitin synthesis in *Saccharomyces cerevisiae*: The *CSD2* gene product is related to chitin synthases and to developmentally regulated proteins in *Rhizobium* species and *Xenopus laevis*. *Mol. Cell. Biol.* **12**: 1764–1776.
3. Bulawa, C. E. and B. C. Osmond. 1990. Chitin synthase I and chitin synthase II are not required for chitin synthesis *in vivo* in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **87**: 7424–7428.
4. Bulawa, C. E., M. Slater, E. Cabib, J. Au-Young, A. Sburlati, W. L. Adair, and P. W. Robbins. 1986. The *Saccharomyces cerevisiae* structural gene for chitin synthase is not regulated for chitin synthesis *in vivo*. *Cell* **46**: 213–225.
5. Cabib, E., A. Sburlati, B. Bower, and S. J. Silverman. 1988. Fungal cell wall synthesis: The construction of a biological structure. *Microbiol. Sci.* **5**: 370–375.
6. Cabib, E. 1991. Differential inhibition of chitin synthases 1 and 2 from *Saccharomyces cerevisiae* by polyoxin D and nikkomycin Z. *Antimicrob. Agents Chemother.* **35**: 170–173.
7. Cabib, E. and V. Farkas. 1971. The control of morphogenesis; an enzymatic mechanism for the initiation of septum formation in yeast. *Proc. Natl. Acad. Sci. USA* **68**: 2052–2056.
8. Castro, A. J. 1967. Antimalarial activity of prodigiosin. *Nature* **4**: 903–904.

9. Choi, W. J. 1998. The determination of chitin synthases by varying pH and divalent cations in *Candida albicans*. *J. Microbiol. Biotechnol.* **8(6)**: 613–617.
10. Choi, W. J., A. Sburlati, and E. Cabib. 1994. Chitin synthase 3 from yeast has zymogenic properties that depend on both the *CAL1* and the *CAL3* genes. *Proc. Natl. Acad. Sci. USA* **91**: 4727–4730.
11. Choi, W. J. and E. Cabib. 1994. The use of divalent cations and pH for the determination of specific yeast chitin synthetases. *Anal. Biochem.* **219**: 368–372.
12. Custro, A. J., A. H. Crowin, F. J. Waxham, and A. L. Beilby. 1959. Products from *Serratia marcescens*. *J. Org. Chem.* **24**: 455–459.
13. Han, S. B., H. M. Kim, Y. H. Kim, C. W. Lee, E. S. Jang, K. H. Son, S. U. Kim, and Y. K. Kim. 1998. T-cell specific immunosuppression by prodigiosin isolated from *Serratia marcescens*. *Int. J. Immunopharmacol.* **20**: 1–13.
14. Hoepflich, P. 1995. Antifungal chemotherapy. *Prog. Drug Res.* **44**: 87–127.
15. Hwang, C.-W., I.-C. Park, W.-H. Yeh, M. Takagi, and J.-C. Ryu. 1997. A partial nucleotide sequence of chitin synthase (*CHS*) gene from rice blast fungus, *Pyricularia oryzae* and its cloning. *J. Microbiol. Biotechnol.* **7**: 157–159.
16. Hwang, E.-I., M.-K. Kim, H.-B. Lee, Y.-K. Kim, B.-M. Kwon, K.-H. Bae, and S.-U. Kim. 1999. Gomisins N and wuweizisus C, new inhibitors for chitin synthase II from *Schizandra chinensis*. *Yakhak Hoeji* **43**: 509–515.
17. Hwang, E.-I., B.-S. Yun, Y.-K. Kim, B.-M. Kwon, H.-G. Kim, H.-B. Lee, K.-S. Bae, and S.-U. Kim. 2000. Chaetoatrosin A, a novel chitin synthase II inhibitor produced by *Chaetomium atrobrunneum* F449. *J. Antibiotics* **53**: 248–255.
18. Jeong, T.-S., E.-I. Hwang, H.-B. Lee, E.-S. Lee, Y.-K. Kim, B.-S. Min, K.-H. Bae, S.-H. Bok, and S.-U. Kim. 1999. Chitin synthase II inhibitory activity of Ursolic acid, isolated from *Crataegus pinnatifida*. *Planta medica* **65**: 261–263.
19. Kang, M. S., N. Elango, E. Mattoa, J. Au-Young, P. W. Robbins, and E. Cabib. 1984. Isolation of chitin synthetase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **259**: 14966–14972.
20. Kim, S.-U., E.-I. Hwang, J.-Y. Nam, K.-H. Son, S.-H. Bok, H.-E. Kim, and B.-M. Kwon. 1999. Inhibition of chitin synthase II by catechins from stem bark of *Taxus cuspidata*. *Planta medica* **65**: 97–98.
21. Kurtz, M. 1988. New antifungal drug targets: A vision for the future. *ASM News* **64**: 31–39.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
23. Mio, T., T. Yabe, M. Sudoh, Y. Satoh, T. Nakajima, M. Arisawa, and H. Yamada-Okada. 1996. Role of three chitin synthase genes in the growth of *Candida albicans*. *J. Bacteriol.* **178**: 2416–2419.
24. Munro, C. A., D. A. Schofield, G. W. Gooday, and N. A. R. Gow. 1998. Regulation of chitin synthesis during dimorphic growth of *Candida albicans*. *Microbiology* **144**: 391–401.
25. Odds, F. 1996. Resistance of clinically important yeasts to antifungal agents. *Int. J. Antimicrob. Agents* **6**: 145–147.
26. Orlean, P. 1987. Two chitin synthases in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **262**: 5732–5739.
27. Rhodes, J., H. Jensen, A. Nilus, C. R. Chitambar, S. G. Farmer, R. G. Washburn, P. E. Steele, and T. W. Amlung. 1992. *Aspergillus* and aspergillosis. *J. Med. Vet. Myc.* **30**: 51–57.
28. Saral, R. 1991. *Candida* and *Aspergillus* infections in immunocompromised patients: An overview. *Rev. Infect. Dis.* **13**: 487–492.
29. Sburlati, A. and E. Cabib. 1986. Chitin synthetase 2, a presumptive participant in septum formation in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **261**: 15147–15152.
30. Silverman, S. J. 1989. Similar and different domains of chitin synthase 1 and 2 of *Saccharomyces cerevisiae*: Two isozymes with distinct functions. *Yeast* **5**: 459–467.
31. Silverman, S. J., A. Sburlati, M. Slater, and E. Cabib. 1988. Chitin synthase 2 is essential for septum formation and cell division in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **85**: 4735–4739.
32. Valdivieso, M. H., P. C. Mol, J. A. Shaw, E. Cabib, and A. Duran. 1991. *CAL1*, a gene required for activity of chitin synthase 3 in *Saccharomyces cerevisiae*. *J. Cell. Biol.* **114**: 101–109.