Differential Sensitivity between Fks1p and Fks2p against a Novel \(\beta\)-1,3-Glucan Synthase Inhibitor, Aerothricin1

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Fks1p and Fks2p are catalytic subunits of \(\beta\)-1,3-glucan synthase, which synthesize \(\beta\)-1,3-glucan, a main component of the cell wall in Saccharomyces cerevisiae. Although Fks1p and Fks2p are highly homologous, sharing 88.1% identity, it has been shown that Fks2p is more sensitive than Fks1p to one of echinocandin derivatives, which inhibits \(\beta\)-1,3-glucan synthase activity. Here we show a similar differential sensitivity between Fks1p and Fks2p to a novel \(\beta\)-1,3-glucan synthase inhibitor, aerothricin1. To investigate the molecular mechanism of this differential sensitivity, we constructed a series of chimeric genes of FKS1 and examined their sensitivity to aerothricin1. As a result, it was shown that a region around the fourth extracellular domain of Fks2p, containing 10 different amino acid residues from those of Fks1p, provided Fks1p aerothricin1 sensitivity when the region was replaced with a corresponding region of Fks1p. In order to identify essential amino acid residues responsible for the sensitivity, each of the 10 non-conserved amino acids of Fks1p was substituted into the corresponding amino acid of Fks2p by site-directed mutagenesis. Surprisingly, only one amino acid substitution of Fks1p (R1336D) conferred Fks1p hypersensitivity to aerothricin1. On the other hand, reverse substitution of the corresponding amino acid of Fks2p (I1355K) resulted in loss of hypersensitivity to aerothricin1. These results suggest that the 1355th isoleucine of Fks2p plays a key role in aerothricin1 sensitivity.

The fungal cell wall consists mainly of \(\beta\)-1,3-glucans, manno-proteins, a small amount of chitin, and several proteins, all of which are interconnected, providing cells their rigidity and protecting them from osmotic pressure (1, 2). As the fungal cell wall is one of the essential architectures for fungal growth and, as mammalian cells do not have such architecture, enzymes that synthesize, assemble, retain, and remodel the fungal cell wall have been thought to be promising targets for antifungal agents (2–4).

\(\beta\)-1,3-Glucan is the most abundant component in the fungal cell wall (2) and is synthesized by \(\beta\)-1,3-glucan synthase (UDPGlucose:1,3-\(\beta\)-d-glucan 3-\(\beta\)-d-glucosyltransferase; EC 2.4.1.34). In Saccharomyces cerevisiae, two kinds of catalytic subunits are encoded by FKS1/GSC1/CWH53/ETG1/CND1/PBR1/YLR342W and FKS2/GSC2/G4074/YGRO32W (5–9). They are highly homologous at the amino acid sequence level, showing 88% identity. Although disruption of either gene alone does not express lethal phenotype, simultaneous disruption of both genes provokes synthetic lethality to the yeast cells (8, 10). These suggest that Fks1p and Fks2p share the function, which is essential for growth. On the other hand, transcriptionally, it is known that their expression is differently controlled; the FKS1 expression is regulated in the cell cycle and predominates during growth on glucose, whereas FKS2 is expressed in the absence of glucose (10). In Candida albicans (11) and Cryptococcus neoformans (12), only one gene encoding the catalytic subunit has been isolated, and it is believed that the genes are essential for their growth because of the lack of success in the establishment of their null mutants. From other fungi, each single gene encoding the catalytic subunit of \(\beta\)-1,3-glucan synthase has been isolated, such as Aspergillus nidulans (13), Aspergillus fumigatus (14), and Paracoccidioides brasiliensis (15). Catalytic subunits from these fungi share the same features, a size greater than 200 kDa and possession of putative 16 transmembrane domains. In addition, Rho1p, a small GTP-binding protein, is known as a regulatory subunit of the \(\beta\)-1,3-glucan synthase in S. cerevisiae (16–18) and C. albicans (19).

Several \(\beta\)-1,3-glucan synthase inhibitors have been identified, such as the echinocandins and the papulacandins (20, 21). Papulacandins are liposaccharide inhibitors isolated from Papularia sphaerosperma. Echinocandins, including cilofungins, aculeacins, mulundocandins, sporiofungins, and pneumocandins, are cyclic hexapeptides with a lipophilic side chain such as linoleoyl or myristoyl moieties. Among these echinocandin derivatives, MK0991 (Merck) has been recently launched, and FK463 (Fujisawa Pharmaceutical Co. Ltd.) and LY303366 ( Lilly) are being developed.

Aerothricin1/RO0093655 is a recently isolated and promising \(\beta\)-1,3-glucan synthase inhibitor produced by Deuteromycotina spp. NR7379 (22–24). This \(\beta\)-1,3-glucan synthase inhibitor is a cyclic lipopeptide-lactone composed of 12 amino acids and a 3′-hydroxypalmitoyl moiety (Fig. 1) and identical to FR901469 (25–27). Aerothricin1 exhibits not only inhibition of C. albicans \(\beta\)-1,3-glucan synthase but also antifungal activity against C. albicans both in vitro culture and in animal models (22–27). However, molecular mechanisms of aerothricin1 inhibition still remain to be clarified.

Here we present a differential sensitivity against aerothricin1 between Fks1p and Fks2p of S. cerevisiae, similar to the characteristics observed with the echinocandin derivative, L-733,560 (6, 10). Furthermore, we identify one determinant amino acid residue involved in this differential sensitivity by using a series of mutant catalytic subunits, Fks1p and Fks2p. Finally, we discuss a possible interaction between aerothricin1 and the catalytic subunit of \(\beta\)-1,3-glucan synthase.

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**EXPERIMENTAL PROCEDURES**

**Media and Strains—**Escherichia coli JM109 and DH5α were used for plasmid amplification, grown at 37 °C in Luria-Bertani (LB) medium with appropriate antibiotics. The *S. cerevisiae* *fks*Δ (8), and their parental strain A451 (MATα, ura3, leu2, trp1, can1, arab7) were cultivated in a medium containing 2% yeast extract, 1% glucose, or 2% dextrose (YPD) or 2% galactose as a carbon source. YOC793 (MATα, ade2, his3, leu2, lys2, trp1, ura3, fis1: HlS3, fis2: LYS2, YCPUGFKS1), originally from YPH499 and kindly provided by Prof. Y. Ohyu, was cultivated in a minimal medium, lacking uracil, and with 2% galactose as a carbon source. Mutant strains constructed in this study were grown in SD medium, lacking l-tryptophan or YPD medium.

**Constructions of Mutants Genes—**Manipulation of DNA was according to standard protocol (28). For the vector, which confers constitutive expression, a PCR-amplified GAP promoter (29), using primers (5'-CCCGGTATCTAGCTTGAATGTTTAG-3') was inserted into pRS414 (Stratagene) as a BamHI- EcoRI fragment, generating pRS414-pT.

Chimeric molecules are shown in Fig. 3. The first series of chimeric genes were constructed by general recombinase techniques. Region A of both FKS1 and FKS2 and a remaining region containing the C-terminal of FKS1 were amplified by PCR. Sequences of primers were 5'-CCCCGAATTCCTATAGCAGTTGAATGTTAG-3' for the region A of FKS1, 5'-CCCCCGATCTGTTATGTTATTTAGTCCC-3' for the region A of both FKS1 and FKS2, and 5'-TTTGCCGCTTCATTACAAATCGAAGTCTGTTATTTAGTCCC-3' for the remaining region containing the C-terminal of FKS1. The amplified fragments were subcloned into pT blue (Takara Ltd.) and subjected to sequencing for confirmation. A remaining region containing a C terminus of FKS2 was prepared as a SalI- ApaI fragment. The A regions and the remaining regions containing C terminus were ligated and inserted into the pRS414-pT as an EcoRI-Apal fragment, generating FKS1-A2 and FKS2-A1 genes.

For FKS1-B2, -C2, and -D2 and FKS2-C2, a technique of splicing overlap extension by PCR (14, 30) was applied. Fragments corresponding to the C-terminal regions B-D of Fks1p and Fks2p were amplified by PCR, generating intermediate products of each of chimeras. For region B of FKS1, 5'-ATTTCAGGATACATACTTTGATC-CC-3' and 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used. For region B of FKS2, 5'-ATTGTCGACAACTACCGTCGAGAAGAC-3' were used.

**Membrane Preparation and Measurement of β-1,3-Glucan Synthase Inhibition—**Membrane preparation and partial purification of β-1,3-glucan synthase were done as previously described (16). β-1,3-Glucan synthase activity measurement was done as reported previously (19). Briefly, membrane fractions were prepared from late log phase cells, and the enzyme was then partially purified by the product entrapment.

**Inhibition of β-1,3-Glucan Synthase by Aerothricin1**

**Fig. 1.** Chemical structure of aerothricin1.
About 30–40 ng of the purified enzymes was incubated in the reaction buffer containing 0.1 mM UDP-[6-3H]glucose (222 Bq, Amersham Biosciences), 75 mM Tris-HCl, pH 7.5, 0.75 mM EDTA, 25 mM KF, 20 μM GTP, 0.1% bovine serum albumin, and 7.8% glycerol in 100 μl at 25 °C for 30 min. After filtration and two steps of washing with 70% ethanol, radiolabeled glucose incorporated into polymerized glucan on the filter was quantified by counting the radioactivity (MicroBeta; Wallac).

RESULTS
Differential Sensitivity to Aerothricin1 between S. cerevisiae fks1 and fks2 Null Mutants—It is known that S. cerevisiae fks1 mutant is more sensitive to L-733,560, one of the echinocandin derivatives, than the wild type strain (6). This differential sensitivity is thought to be due to biochemical characteristics of Fks2p, which is more sensitive to this compound than Fks1p (10). These lines of evidence intrigued us to test the antifungal activity of aerothricin1 (Fig. 1), a novel -1,3-glucan synthase inhibitor, against both fks1Δ and fks2Δ null mutants.

As shown in Table I, the fks1Δ null mutant appeared to be more sensitive to aerothricin1 than the fks2Δ null mutant or the parental wild type strain A451. In an attempt to address this differential sensitivity more precisely, each of the FKS1 and FKS2 expression plasmids was introduced into the fks1Δ fks2Δ double null mutant harboring the URA3-borne GALp-FKS1 plasmid, and this plasmid was then eliminated by 5-fluoroorotic acid treatment. In the result-ant cells, either the FKS1 or FKS2 gene can be expressed in the absence of endogenous Fks1p and Fks2p under the control of a constitutive GAP promoter (29). The introduction of either

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\text{TABLE I}
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Aerothricin1 sensitivity of S. cerevisiae fks null mutants

Growth inhibition was measured by microdilution assay. 10⁴ cells of fks null mutants were cultivated with 100 μl of medium on 96-well microtiter plates at 30 °C for 16–24 h in the presence or absence of antifungal compounds. Values represent the mean IC₅₀ values, referring to the compound concentrations that gave 50% inhibition of cell growth compared with the control, in three experiments and ± S.D.

| Genotype of host cell | Plasmid       | Expressed Fksp | Aerothricin1 | Cycloheximide |
|-----------------------|---------------|----------------|-------------|--------------|
| Genotype of host cell | Plasmid       | Expressed Fksp | Aerothricin1 | Cycloheximide |
| Wild type             | None          | Fks1p, Fks2p   | 0.12 (± 0.0050) | 0.030 (± 0.0012) |
| fks1                  | None          | Fks2p          | 0.0033 (± 0.0011) | 0.027 (± 0.0058) |
| fks2                  | None          | Fks1p          | 0.16 (± 0.0036)  | 0.028 (± 0.0017) |
| fks1, fks2            | GAPp-FKS1     | Fks1p          | 0.39 (± 0.068)   | 0.045 (± 0.0012) |
| fks1, fks2            | GAPp-FKS2     | Fks2p          | 0.029 (± 0.0032) | 0.038 (± 0.0013) |

A

![Fig. 2. Aerothricin1 sensitivity of GAPp-FKS1 and GAPp-FKS2 on a spotting assay.](image)

10⁴ cells expressing each FKS1 and FKS2 under the control of GAP promoter were spotted onto YPD plates containing different concentrations of aerothricin1. After overnight incubation at 30 °C, MICs of aerothricin1 were determined as minimum concentrations inhibiting the cell growth.

B

![Fig. 3. Chimeric mutants of FKSs.](image)

A, predicted structures of Fks1p and Fks2p with regions used for replacement between Fks1p and Fks2p are illustrated. Hatched boxes indicate putative transmembrane regions. B, MICs of aerothricin1 against chimeric proteins were determined with a spotting assay. 10⁴ cells were spotted onto YPD agar plates containing different concentrations of aerothricin1 from 0.003 to 1 μg/ml. After overnight incubation at 30 °C, MICs of aerothricin1 were determined as minimum concentrations inhibiting the cell growth. Filled and open boxes indicate regions of Fks1p and Fks2p, respectively. In the Type of sensitivity column, Fks1p means that its MIC value is equal to or more than 0.3 μg/ml, and Fks2p means equal to or less than 0.03 μg/ml.
GAPp-driven FKS1 or GAPp-driven FKS2 suppressed the lethal phenotype of S. cerevisiae fks1Δ fks2Δ double null mutant, and the resulting mutant showed the same growth rate as the parental wild type strain, YPH499 (data not shown).

At first, we confirmed the differential sensitivity between the two strains. As shown in Table I, the double null mutant expressing only Fks2p was more sensitive to aerothricin1 than that expressing only Fks1p. We also questioned whether they showed a differential sensitivity against another type of anti-fungal agent, cycloheximide. However, we observed no clear difference in their sensitivities against this agent (Table I). These results suggest that this differential sensitivity against aerothricin1 may simply rely on differences between Fks1p and Fks2p.

Identification of Regions Containing Determinant(s) for the Aerorthricin1 Sensitivity of Fks2p—Based on the hypothesis that differences in the primary sequences of Fks1p and Fks2p may represent determinants for the aerothricin1 sensitivity, we first looked at the intracellular domain at the N terminus of Fks2p because this region is less homologous, even though Fks1p and Fks2p exhibit 88.1% identity throughout overall sequences. For this purpose, two kinds of chimeric genes, FKS1-A2 and FKS2-A1, were constructed by replacing the N-terminal region (Fig. 3, A and B) and introduced into the fks2Δ double null mutant under the control of the GAP promoter. For a rapid profiling of their sensitivities, we applied a spotting assay with plates containing different concentrations of aerothricin1. Although aerothricin1 sensitivities were determined as MICs in this assay (see “Experimental Procedures” and Fig. 2), we could see the same differential sensitivity as seen in the comparison of IC50 values against the double null mutant cells expressing either Fks1p or Fks2p (Table I). Both chimeric proteins, Fks1-A2p and Fks2-A1p, appeared to suppress the synthetic lethal phenotype of fks1Δ fks2Δ double null mutant because no growth defects observed compared with the parent strain YPH499 (data not shown). As shown in Fig. 3B, the spotting assay revealed that Fks1-A2p failed to confer the mutant cells hypersensitive to aerothricin1. Surprisingly, the mutant cells expressing Fks2-A1p showed Fks2p-like sensitivity. These results indicate that the determinant(s) may exist in the C-terminal region of Fks2p, which is highly conserved between Fks1p and Fks2p, sharing 92.5% identity.

To minimize regions containing the determinant(s), we performed the second round of chimeric gene analysis. As illustrated in Fig. 3A, the sequence encoding the C-terminal region of Fks2p was divided into three regions, named A, B, and C. Each of them was replaced with the corresponding region of FKS1 gene, resulting in chimeric genes FKS1-B2, FKS1-C2, and FKS1-D2. Mutant cells harboring each chimeric gene also grew normally (data not shown). By the spotting assay, it was shown that only Fks1-C2p conferred the mutant cells hypersensitive to aerothricin1 (Fig. 3B). We also tested an opposite substitution, Fks2-C1p, in which the region C of Fks2p was replaced with that of Fks1p (Fig. 3A). Interestingly, the replacement of region C in Fks2p resulted in a loss of hypersensitivity to aerothricin1 (Fig. 3B), suggesting that region C of Fks2p contains the determinant(s) of differential sensitivity to aerothricin1.

To localize a region containing the determinant(s) more precisely, we further divided a portion of Fks2p including region C into three parts (region E, F, G in Fig. 3A) and constructed chimeric genes by replacing the cognate region in FKS1 with that of FKS2 gene. Finally, it was found that an introduction of region E of Fks2p into Fks1p was enough to provide the aerothricin1 sensitivity to the mutant cells (Fig. 3B, FKS1-E2). Conversely, Fks2p harboring a replacement of region E failed to confer the mutant cells sensitive to the inhibitor (Fig. 3B, FKS2-E1).

The 1355th Ile Is Essential for the Aerorthricin1 Sensitivity of Fks2p—From a series of analyses using chimeric proteins, it was suggested that determinant(s) could be located within a region shared by regions C and E (Fig. 4A). The shared region consists of 74 amino acids and contains 10 non-conservative amino acids between Fks1p and Fks2p (86.5% identical). Identification of these non-conservative amino acids prompted us to question which amino acid was essential for the aerothricin1 sensitivity of Fks2p. For this purpose, we mutagenized each of them in Fks1p with that of Fks2p by using site-directed mutagenesis. All 10 Fks1 mutant proteins were analyzed in the fks1Δ fks2Δ double null mutant cells with the spotting assay. Surprisingly, as summarized in Fig. 4B, only one mutant Fks1p (FKS1K1336I) conferred the cells sensitive to aerothricin1. We also examined the effects of substitution of the corresponding amino acid residue of Fks2p with that of Fks1p and found that this opposite substitution resulted in a complete loss of the aerothricin1 hypersensitivity (Fig. 4B, FKS2I1355K). The switching of the sensitivity due to these substitutions was further confirmed by determination of IC50 values of aerothricin1 against the fks1Δ fks2Δ double null mutant cells expressing each mutant protein (Table I). Although IC50 values of echinocandin B against these cells were also determined, no clear difference was observed in their sensitivities.

Next we investigated the effects of substitutions on biochemical properties of Fks1p and Fks2p. β-1,3-Glucan synthase complexes containing the mutant catalytic subunits were partially purified from the fks1Δ fks2Δ double null mutant cells
Inhibition of β-1,3-Glucan Synthase by Aerothricin1

**Table II**

| Plasmid | Growth inhibition | Glucan synthase activity | Aerothricin1 IC50 |
|---------|------------------|--------------------------|------------------|
|         | Aerothricin1 IC50 | Echinocandin B IC50      | Specific activity | Aerothricin1 IC50 |
|         | µg/ml            | µg/ml                    | pmol/mg protein/h | µg/ml            |
| GAPp-FKS1 | 0.39 (± 0.068)   | 1.5 (± 0.061)            | 64 (± 5.0)       | 5.3 (± 1.9)      |
| GAPp-FKS811336 | 0.052 (± 0.020) | 3.0 (± 0.038)            | 79 (± 2.8)       | 0.091 (± 0.010)  |
| GAPp-FKS2 | 0.029 (± 0.0032) | 1.1 (± 0.15)             | 39 (± 4.5)       | 0.099 (± 0.011)  |
| GAPp-FKS211355K | 0.66 (± 0.029) | 0.45 (± 0.027)           | 22 (± 2.8)       | 1.1 (± 0.15)     |

**Fig. 5. The fourth extracellular domain of glucan synthase catalytic subunits from various fungi.** The amino acid sequences of *C. neoformans* Fks1p and Fks2p from residues 1317–1357 and 1336–1376, respectively, are aligned to the sequences of Fks proteins from *C. albicans* (CaGsc1), *A. fumigatus* (AfFKs), *A. nidulans* (AnFKs), *P. brasiliensis* (PbFKs), and *C. neoformans* (CnFKs). Filled boxes indicate conserved and high conserved residues among seven catalytic subunits of β-1,3-glucan synthase. Filled boxes indicate determinant amino acid residues.

It has been shown that aerothricin inhibits in vitro β-1,3-glucan synthase of *C. albicans* and growth of various fungi, such as several *Candida* species and *A. fumigatus* (22–27), the detailed molecular mechanisms of the inhibition are still unknown. In this report, we found that the fks1 null mutant was more sensitive to aerothricin1 than the fks2 null mutant or the parental strain in *S. cerevisiae*. This observation is the first evidence suggesting that the catalytic subunit of β-1,3-glucan synthase would be a molecular target of aerothricin1. In the course of our experiments shown here, we initially used a number of chimeric Fks proteins. Surprisingly, none of them resulted in impaired growth when expressed in the fks1Δ fks2Δ double null mutant of *S. cerevisiae*. These results indicate not only that these chimeric proteins are functional but also that Fks1p and Fks2p are highly structurally homologous. Although we cannot exclude a possibility that other amino acid residues are involved in the aerothricin1 sensitivity of Fks2p, several lines of evidence presented here demonstrate that one amino acid residue, Ile-1335 of Fks2p, is one dominant determinant for its aerothricin1 sensitivity. Alternatively, Lys-1336 of Fks1p is the dominant one for the resistance to aerothricin1. One possible explanation of these determinant residues in the interaction with aerothricin1 is that their charges may affect affinity between Fks proteins and aerothricin1; a positive charge of the 1336th lysine residue of Fks1p may interfere with the interaction of aerothricin1 with Fks1p molecules, because aerothricin1 has a positive-charged nitrogen at the ornithine moiety (Fig. 1), which is essential for its inhibitory activity (data not shown). Alternatively, the hydrophobicity of the 1355th isoleucine residue of Fks1p may be important for the aerothricin1 association.

Aerothricin1 exhibits growth inhibition effectively against at least *C. albicans* and *A. fumigatus* (24, 25); the IC50 values against *C. albicans* ATCC48130 and *A. fumigatus* CF1003 were 0.03 and 0.06 µg/ml, respectively. As shown in Fig. 5, primary structures of the fourth extracellular domains, including the determinant residue, are conserved among these fungi. Interestingly, positions of the expected determinant are occupied with isoleucine or valine residues, which are non-charged and hydrophobic, supporting the importance of the hydrophobic residue of Fks2p for aerothricin1 interaction. It is interesting to question whether *A. nidulans* and *P. brasiliensis* are sensitive to this compound because the region including the determinant residue is also highly conserved and possesses the determinant isoleucine residue. *C. neoformans* is known to be less sensitive to aerothricin1 in growth inhibition assay (25) even though we can find an isoleucine residue in its Fks1p at the same position when aligned with Fks proteins from other sensitive fungi (Fig. 5). However, its sequence similarity is quite low against other Fks proteins, suggesting the region is structurally different from other Fks proteins.

Echinocandins share similar features with aerothricin1 in their chemical structure, such as cyclic macropeptides with a lipophilic side chain. In particular, *S. cerevisiae* Fks1p and Fks2p exhibit differential sensitivities against both types of inhibitors. Therefore, it is possible that they may share domains that interact with Fks proteins. However, it is unlikely that they share the same determinant(s) for their differential sensitivities because we failed to find any clear differences in the sensitivities of point-mutated Fks proteins (Table II). In addition, Fks1-A2p was more sensitive to echinocandin B than Fks1p, Fks2p, or Fks2-A1p (data not shown). These observations suggest that aerothricin1 and the echinocandins may interact differently with catalytic subunits via different determinant residue(s).

By investigating the differential aerothricin1 sensitivity between Fks1p and Fks2p, we obtained a clue for understanding the mechanism of inhibition of β-1,3-glucan synthase by aerothricin1. For further analysis of the aerothricin1 inhibition, focus should be on the fourth extracellular domain. Monitoring a direct interaction between aerothricin1 and the fourth extracellular domain might be useful in understanding the actual
physical relationship between aerothricin1 and the catalytic subunits of β-1,3-glucan synthase. These results would be helpful for developing more potent derivatives from aerothricin1.

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