## Review

# Immunochemistry of pathogenic yeast, Candida species, focusing on mannan

By Nobuyuki Shibata,[*1] Hidemitsu Kobayashi[*2] and Shigeo Suzuki[*3,*4,†]

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**Abstract:** This review describes recent findings based on structural and immunochemical analyses of the cell wall mannan of *Candida albicans*, and other medically important *Candida* species. Mannan has been shown to consist of $\alpha$-1,2-, $\alpha$-1,3-, $\alpha$-1,6-, and $\beta$-1,2-linked mannopyranose units with few phosphate groups. Each *Candida* species has a unique mannan structure biosynthesized by sequential collaboration between species-specific mannosyltransferases. In particular, the $\beta$-1,2-linked mannose units have been shown to comprise a characteristic oligomannosyl side chain that is strongly antigenic. For these pathogenic *Candida* species, cell-surface mannan was also found to participate in the adhesion to the epithelial cells, recognition by innate immune receptors and development of pathogenicity. Therefore, clarification of the precise chemical structure of *Candida* mannan is indispensable for understanding the mechanism of pathogenicity, and for development of new antifungal drugs and immunotherapeutic procedures.

**Keywords:** *Candida*, mannan, ^{1}H NMR, mannosyltransferase, pathogenicity, Dectin-2

## 1. Introduction

*Candida* species causes a variety of infections ranging from superficial candidiasis to life-threatening invasive candidiasis, also known as disseminated or systemic candidiasis. Predisposing factors for candidiasis include: immunosuppression, broad-spectrum antibiotics, and cytotoxic therapies; the presence of intravenous catheters; diabetes; neutropenia; very low birth weights and AIDS.[1] Invasive candidiasis is on the rise with the increasing population of susceptible individuals, while treatment is hampered by antifungal resistance. Thus, *Candida* species have now become the third most prevalent cause of bloodstream infections. *Candida* pathogenicity also depends on hypothetical virulence factors. These include the production of secreted hydrolytic enzymes, dimorphic transition from yeast to mycelium, antigenic variability, phenotype switching, adhesion to host cells, and cell-surface hydrophobicity.

The outermost layer of the cell wall plays an essential role in host interaction, including the triggering and modulation of the anti-*Candida* host immune responses, which appear to rely on the interplay between the innate and adaptive immunities. For this reasons, the cell wall of *Candida* species has been the focus of attention. The outer layer of the cell wall of *Candida* species consists of mannoproteins containing O-glycosylated oligosaccharide and N-glycosylated polysaccharide moieties. Both carbohydrate moieties have been shown to be important in host-fungal interactions and virulence. The N-glycosylated polysaccharide, mannan, has a comb-like structure with an $\alpha$-1,6-linked backbone moiety and many oligomannosyl side chains with a low number of phosphate groups. Some oligosaccharides are connected to a phosphate group by a phosphodiester bond. Phosphate-bound oligosaccharides can be selectively released from mannan by treatment with a weak acid solution, such as 10 mM HCl at 100°C for

*1 Department of Infection and Host Defense, Tohoku Pharmaceutical University, Miyagi, Japan.
*2 Department of Microbiology, Nagasaki International University, Nagasaki, Japan.
*3 Professor Emeritus, Tohoku Pharmaceutical University, Miyagi, Japan.
*4 Sendai Research Institute for Mycology, Miyagi, Japan.
† Correspondence should be addressed: S. Suzuki, 1-14-34 Toshogu, Aoba-ku, Sendai, Miyagi 981-0908, Japan (e-mail: suzusige@seagreen.ocn.ne.jp).
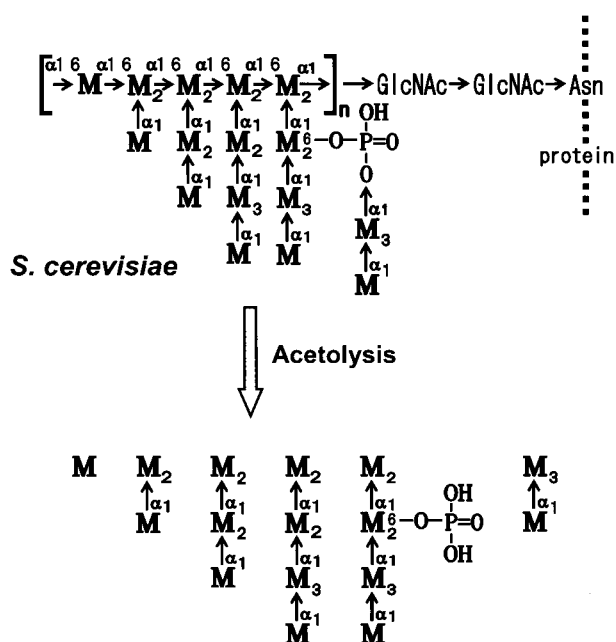Abbreviations: ManTase: mannosyltransferase; PA: pyridylamino.

Fig. 1.  Structure of the cell-wall mannan of *S. cerevisiae*. M denotes an α-D-mannopyranose unit. The acetolysis cleaves the α-1,6-linkage of the mannan to produce side chain oligosaccharides.

1 h. Mannan can thus be fragmented by acetolysis, which selectively cleaves the backbone α-1,6-linked mannose units (Fig. 1). The chemical structure of the resultant mannooligosaccharides that originate from the side chains have been analyzed by methylation, and $^1$H and $^{13}$C NMR, and the antigenic properties studied by inhibition assays in mannan-anti-mannan antibody systems. Mannan displays strong antigenicity, dominating the humoral antibody response, and is also recognized by the innate immune system. Therefore, determination of the chemical structure of mannan of medically important pathogenic *Candida* species is indispensable in order to elucidate the bio-defense mechanisms of hosts as well as for identifying the mechanisms of pathogenicity.

## 2. Structural and serological studies of *Candida* mannan in early stage

Comprehensive antigenic analyses of the genus *Candida* were first carried out by Tsuchiya *et al.*[2)–4)] and Fukazawa *et al.*[5)] on *C. albicans*, *C. tropicalis*, *C. stellatoidea*, *C. parapsilosis*, *C. guilliermondii*, *C. glabrata*, *C. krusei*, and *C. kefyr*. All of the reciprocally adsorbed antisera were examined by a slide agglutination test and the antigenic formulas were then formulated. Hasenclever and Mitchell[6)]

proposed that the *C. albicans* species could be divided into two serotypes, A and B. Later, Summers *et al.*[7)] showed that the chemical entity of the major antigen of the genus *Candida* was a cell wall polysaccharide, mannan. Gorin and Spencer[8)] extracted mannose-containing polysaccharides from hundreds of species of fungi and analyzed them by $^1$H NMR. A β-1,2-linkage containing mannan was first reported by Gorin *et al.*[9)] in *Pichia pastoris* and *Citeromyces matritensis*. We demonstrated the usefulness of the precipitin-inhibition assay using mannooligosaccharides obtained from mannan by acetolysis for the structural identification of epitopes,[10),11)] and found that mannohexaoses, Manα1-2Manα1-2Manα1-2Manα1-2Manα1-2Man and Manα1-3Manα1-2Manα1-2Manα1-2Manα1-2Man, are the strongest epitopes for the *C. albicans* serotype A[12)] and B mannan.[13)] Suzuki and Fukazawa[14)] also showed the antigenicity of the mannohexaose, Manα1-3Manα1-2Manα1-2Manα1-2Manα1-2Man, in *C. albicans* serotype A mannan. Funayama *et al.*[15),16)] reported the presence of a mannohexaose side chain, Manα1-2Manα1-3Manα1-2Manα1-2Manα1-2Man, in *C. albicans* serotype B and *C. parapsilosis* mannan. The presence of structural and immunochemical heterogeneity in mannan was shown by Okubo *et al.*[17),18)] using stepwise anion exchange chromatography. They showed that the reactivity of mannan subfractions against anti-mannan serum correlates well with the amount of phosphate groups and phosphodiesterified oligosaccharides. The structure of antigenic factors of mannan in medically important *Candida* species have been described elsewhere.[19)–21)] It is noteworthy that the use of high-resolution NMR spectroscopy made possible great advances in the immunochemistry of *Candida* mannan.

## 3. β-1,2-Linked mannooligosaccharides connected via a phosphate group of the mannan side chain

Mannan of *S. cerevisiae* contains α-1,3-linked mannobiose bound through phosphate groups in the side chain.[22)] However, we found that β-1,2-linked oligomannosyl units up to heptaose, Manβ1-2Manβ1-2Manβ1-2Manβ1-2Manβ1-2Manβ1-2Man, exist in the antigenic mannan of *C. albicans* in an acid-labile phosphodiesterified form.[23),24)] The β-1,2-linked mannooligosaccharide moieties were found to display stronger antigenicity than the α-linked ones in the humoral antibody response of mammals,[25)] corresponding to antigenic epitope, factor 5.[26)]
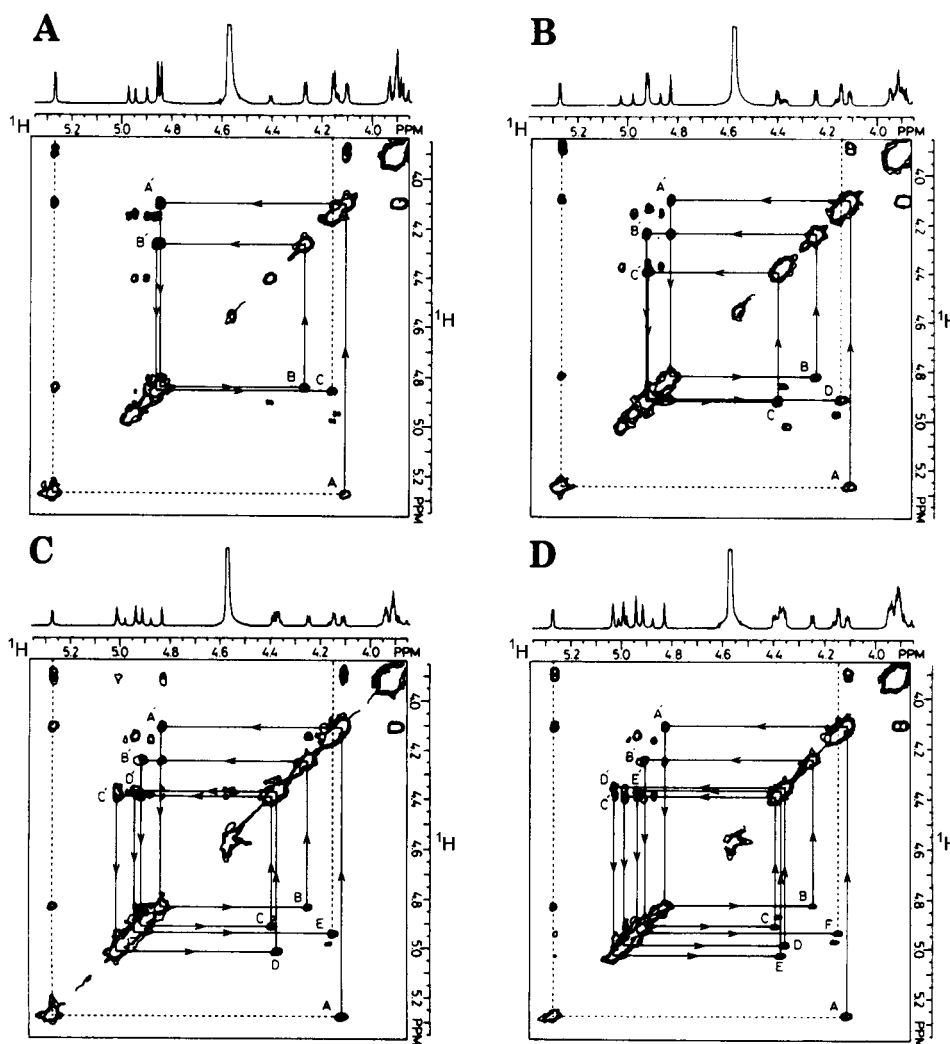
Fig. 2. Sequential H-1-H-2′ connectivities of mannose units of the $\beta$-1,2-linked mannooligosaccharide (Ref. 27). (A) Mannotriose; (B) Mannotetraose; (C) Mannopentaose; (D) Mannohexaose. The right side of the diagonal shows COSY, and the left side of the diagonal shows NOESY. Primed letters indicate inter-unit H-1-H-2′ NOE cross-peaks and unprimed letters, the H-1-H-2-correlated cross-peaks due to $J$-coupling. Arrows indicate the direction of the sequential connectivity from the reducing terminal unit to the nonreducing terminal unit.

The H-1 and H-2 signals of the $\beta$-1,2-linked mannooligosaccharides isolated by mild acid hydrolysis were assigned by a sequential NMR method that combines two-dimensional (2D) $^1$H-$^1$H correlated spectroscopy (COSY) and 2D nuclear Overhauser effect (NOE) spectroscopy (NOESY) (Fig. 2).[27] The results indicate that H-1 and H-2 of each $\beta$-1,2-linked mannose unit show significantly different signals compared to those of the $\alpha$-linked units (Fig. 3). Reduction of the reducing terminal of these oligosaccharides by NaBH$_4$ causes a significant downfield shift in the H-1 signals for the second and third mannose units, and an upfield shift for the fourth

mannose unit. This result indicated that the influence of reducing terminal mannose unit reaches as far as the fourth mannose unit from the reducing terminal. The unprecedented shift effect of the H-1 signal suggests a folded conformation. We further assigned all of the $^1$H and $^{13}$C signals in each oligosaccharide.[28] Poulain and coworkers[29),30)] made the same assignments. Nitz $et\ al.$[31] analyzed the solution conformation of the synthetic $\beta$-propyl glycoside derivatives of the $\beta$-1,2-linked mannooligosaccharide and detected many inter-unit NOEs between the n and n+3 units. They also performed a simulated annealing from 500 K high temperature-molecular
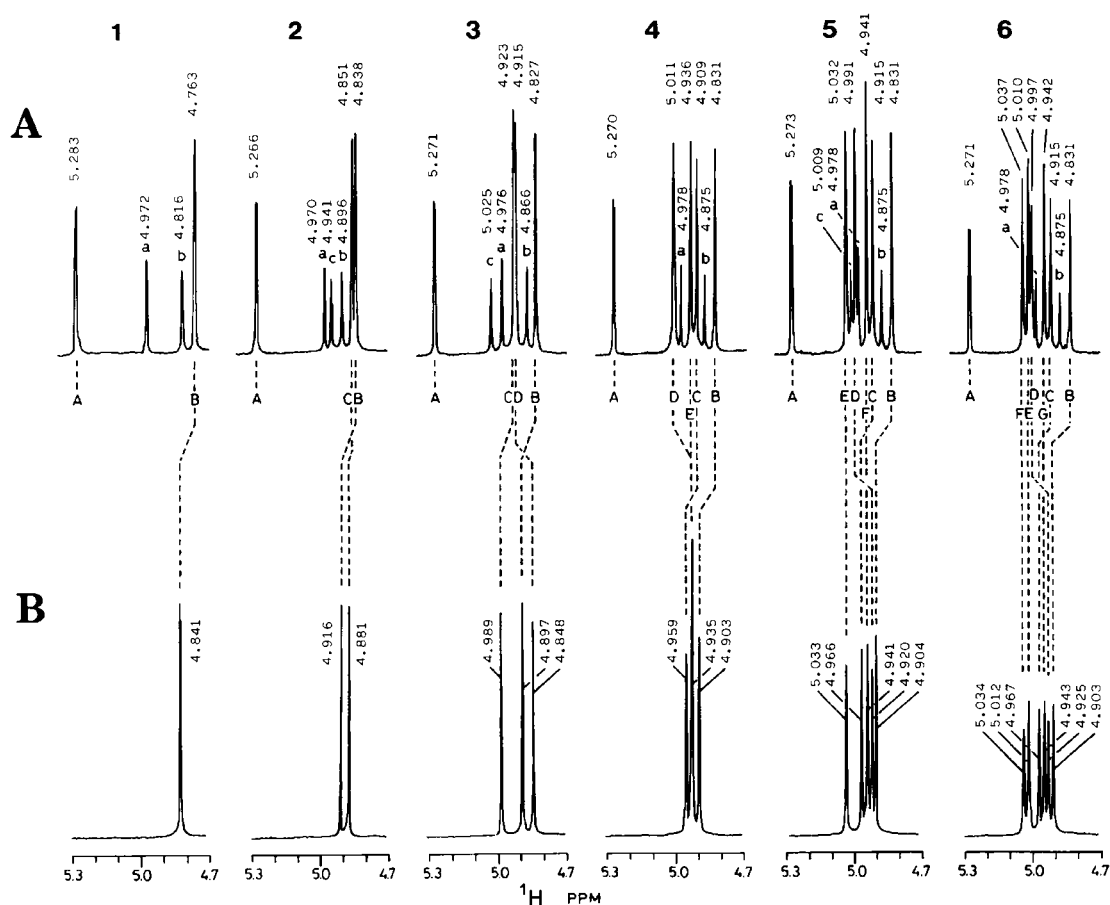
Fig. 3.  $^1$H NMR spectra of the $\beta$-1,2-linked mannooligosaccharides (A) and their corresponding alcohols (B) (Ref. 27). (1) Mannobiose; (2) Mannotriose; (3) Mannotetraose; (4) Mannopentaose; (5) Mannohexaose; (6) Mannoheptaose. The capital letters from A to G and the small letters from a to c refer to the mannose units from the reducing terminal $\alpha$-anomer unit and $\beta$-anomer unit of each oligosaccharide, respectively.

dynamics. The generated random energy structures were separately cooled to 5 K, and then minimized. Based on the results, they concluded that the conformation is a compact helical structure with a three-unit repeat. We also analyzed the conformation of the free $\beta$-1,2-linked mannooligosaccharides and confirmed the compact helical conformation as shown in Fig. 4.[32]

### 4. $\beta$-1,2-Linked mannose units directly connected to the mannan side chain

The side chain of the cell wall mannan from *C. albicans* serotype A strain was fragmented by acetolysis[33] under mild conditions,[34] and separated by Bio-Gel P-2 column chromatography or by high-performance liquid chromatography (HPLC). Although $\alpha$-mannosidase treatment of the acetolysis product of the *C. albicans* serotype B mannan com-

pletely hydrolyzed it to mannose, that of the serotype A mannan retained a large amount of the longer oligosaccharides, from pentaose to octaose.[35]–[38] These oligosaccharides consisted of one to four $\beta$-1,2-linked mannose units connected to the $\alpha$-1,2-linked mannotetraose, Man$\beta$1-[2Man$\beta$1]$_n$-2Man$\alpha$1-2Man$\alpha$1-2Man$\alpha$1-2Man (n = 0 to 3), and corresponded to the serotype A-specific antigen, factor 6.[39] We also determined the structure of mannan in the medically important *Candida* species, *C. tropicalis*,[40] *C. lusitaniae*,[41] and *C. glabrata*,[42] and confirmed the presence of the factor 6 structure. Furthermore, we found that some *Candida* species, *C. guilliermondii*[43] and *C. saitoana*,[44] also contain $\beta$-1,2-linked mannose units. However, the $\beta$-1,2-linked mannose units in these *Candida* mannan were connected to the $\alpha$-1,3-linked mannose units of the side chain. We also found that the side chain
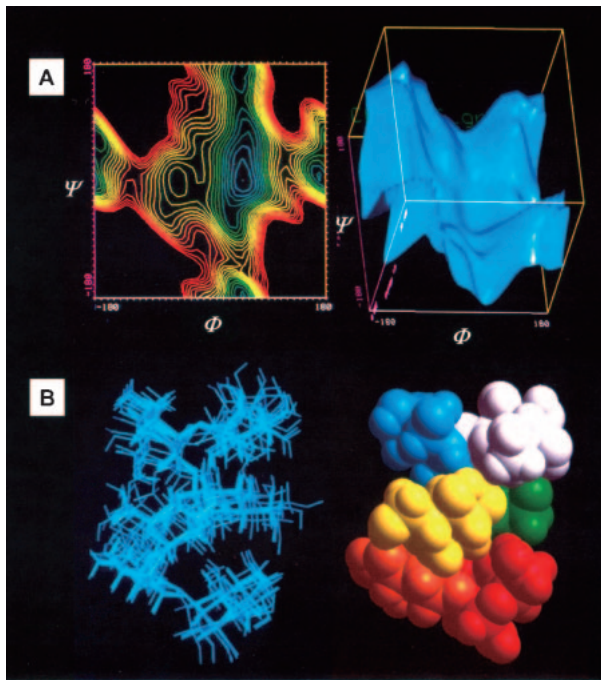
Fig. 4. Conformation analysis of the β-1,2-linked mannooligo-saccharides. (A) Relaxed-residue steric energy map of the β-1,2-linked mannobiose as a function of the Φ (H-1-C-1-O-1-C-2′) and Ψ (C-1-O-1-C-2′-H-2′) torsion angles. (B) Lowest energy conformers of the β-1,2-linked mannohexaose obtained by simulated annealing from 900 K molecular dynamics.

oligosaccharide contains the α-1,6-linked branching mannose unit. This side chain oligosaccharide, Manβ1-2Manβ1-2Manα1-3(Manα1-6)Manα1-2Man, displays different antigenicity from the factor 6, and corresponds to factor 9. As shown in Fig. 5, these *Candida* species were found to contain species-specific mannan structures.

## 5. Additivity rule for the ¹H NMR signals of mannose units in *Candida* mannan

We found an additivity rule for the β-1,2-linked mannose unit.[38] Figure 6A shows the 2D total correlation spectroscopy (TOCSY) spectrum of the *C. albicans* serotype A mannan. The shifts in the H-1-H-2-correlated cross-peaks caused by the addition of the α-1,2-linked mannose unit and the β-1,2-linked mannose unit are shown by the solid arrows and the dashed arrows, respectively. The shift caused by the addition of the β-1,2-linked mannose unit was significantly different from that caused by the addition of the α-1,2-linked mannose unit. The addition of the β-1,2-linked mannose unit to the nonreducing terminal α-1,2-linked mannose unit of the side chain caused a downfield shift of the latter's cross-peak from 10 to 15. Similar shifts were also observed for the cross-peak of the nonreducing terminal α-1,3-linked mannose unit from 7 to 13.
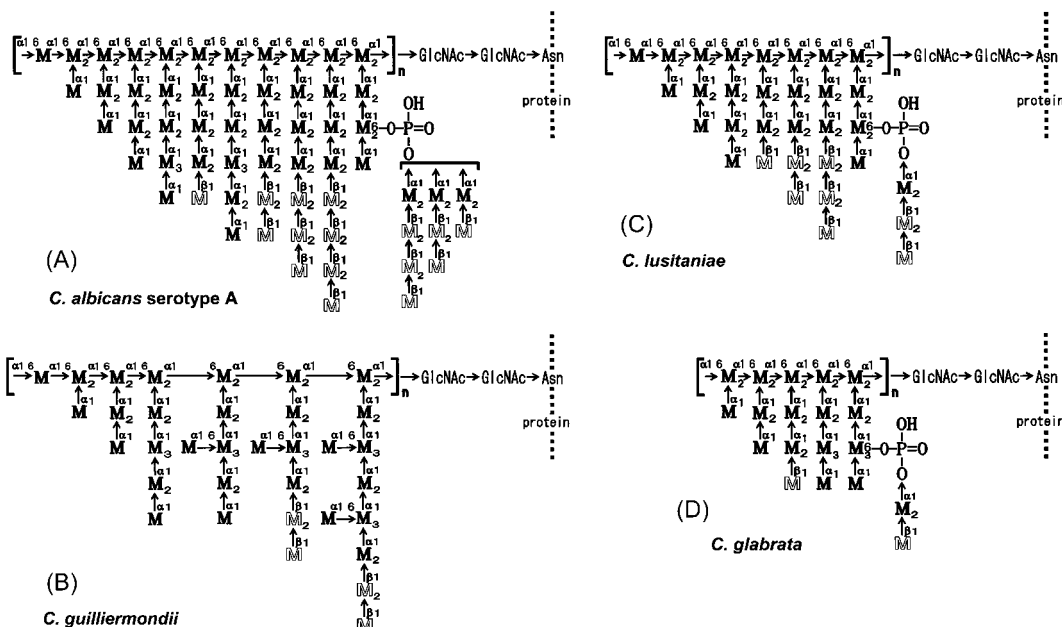


Fig. 5. Structure of the cell wall mannan of *Candida* species. (A) *C. albicans* serotype A, (B) *C. guilliermondii*, (C) *C. lusitaniae*, (D) *C. glabrata*. M set in outlined type indicates β-D-mannopyranose unit. These mannan contain the β-1,2-linked mannose units at the nonreducing terminal side of the side chains as well as at the phosphodiesterified oligosaccharide moiety.
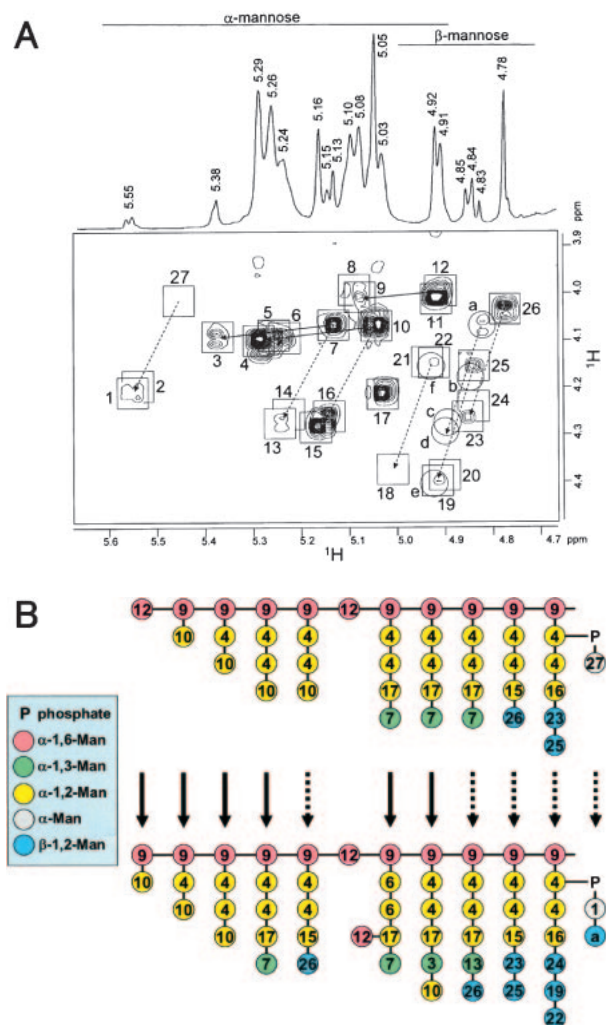
Fig. 6. Additivity rule of the ¹H-NMR chemical shift for mannose units. (A) TOCSY spectrum of the mannan of *C. albicans* serotype A (Ref. 38). The boxed regions in the spectrum indicate the H-1-H-2-correlated cross-peaks of the α- and β-mannose units in the acid-stable polysaccharide moiety. The circled cross-peaks correspond to the β-1,2-linked mannose units of the phosphodiesterified acid-labile oligosaccharide side chains. The broken- and continuous-line arrows indicate the shifts in the cross-peaks caused by the addition of β-1,2-linked mannose units and α-1,2-linked mannose units, respectively. (B) Structural diagram of the mannose units in the mannan and the shift effect of the cross-peaks caused by the addition of mannose units. The numbers on the mannose units correspond to the cross-peaks in panel A.

Figure 6B illustrates the shift effect of the cross-peaks of the side chain mannose units by the addition of an α- or β-mannose unit observed in the TOCSY spectrum of Fig. 6A. These shifts were also found on the nonreducing terminal β-mannose units of the side chains. Cross-peaks 25 and 26, corresponding

to the nonreducing terminal β-1,2-linked mannose units of the serotype A-specific side chain oligosaccharides, caused a downfield shift to 19 and 23, respectively, by the addition of a further β-1,2-linked mannose unit.

## 6. The presence of branched side chains in mannan

Although the presence of a branching structure in *Candida* mannan has been suggested[5),14),45),46)] the branched oligosaccharide has not been identified. We demonstrated the presence of the 3,6-branched hexaose side chain in the mannan of *C. albicans* serotype B by acetolysis under mild conditions, followed by methylation and ¹H NMR analyses, and proposed the chemical structure shown in Fig. 7.[47)] The ¹H NMR signal of the 3,6-branched hexaose showed a characteristic upfield chemical shift on its α-1,2-linked mannose unit. A steric effect of the α-1,6-linked branching mannose unit is causative of the shift of the cross-peak. The *C. stellatoidea* mannan was also found to contain 3,6-branched side chains.[48)] On the other hand, the side chains of the mannan of *C. parapsilosis* consist of linear oligosaccharides.[47)] The presence of an α-1,6-branched mannose unit in the yeast mannan was first determined in the O-linked oligosaccharide[49)] of the mannan of *Saccharomyces kluyveri*, and later in the N-linked mannan moiety.[50)]

## 7. Substrate specificity of mannosyltransferases of *Candida* species

As substrate for the mannosyltransferase (ManTase) assay, mannooligosaccharides were connected to 2-aminopyridine to produce pyridylamino (PA)-derivatives. Using the PA-oligosaccharides as substrate, we assayed ManTase activity in the enzyme fraction of *Candida* cells in the presence of GDP-mannose. The enzyme reaction products were detected by HPLC equipped with a fluorescence spectrometer. To assess the substrate specificity of α-1,6-ManTase of the *C. albicans* serotype B, the PA-derivatives of the several acceptor oligosaccharides prepared from the mannan of *C. albicans*, *C. parapsilosis*, *C. guilliermondii*, *C. krusei*, and *S. cerevisiae* were tested. The result indicates that the oligosaccharides containing α-1,3-linked mannose units at the non-reducing terminal can serve as acceptors for α-1,6-ManTase.[51)] The *C. albicans* serotype A-specific oligosaccharide side chain is biosynthesized by the transfer of the first β-1,2-linked mannose unit to the α-1,2-linked manno-
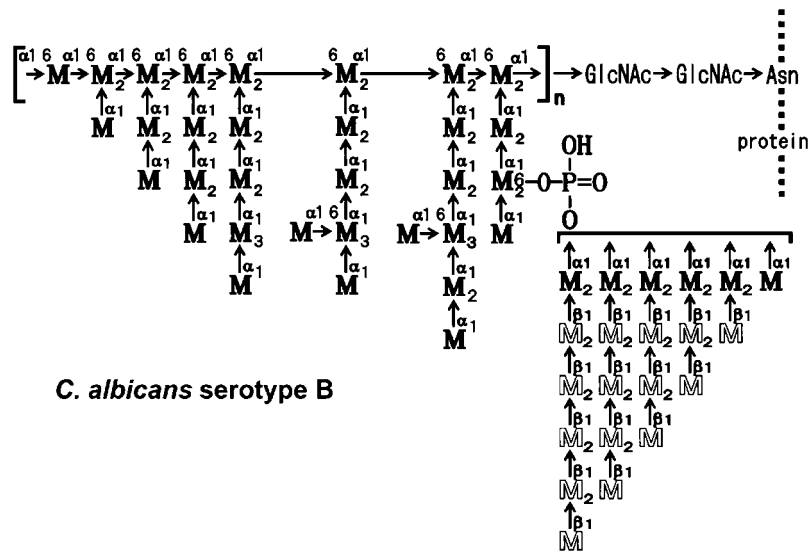
**C. albicans serotype B**

Fig. 7. Structure of the cell wall mannan of *C. albicans* serotype B. This mannan contains 3,6-branched mannose units at the side chains. β-1,2-linked mannose units are present only on the phosphodiesterified oligosaccharide moiety.

tetraose side chain by β-1,2-ManTase I to produce Manβ1-2Manα1-2Manα1-2Manα1-2Man. Next, the second β-1,2-linked mannose unit is transferred by β-1,2-ManTase II to produce Manβ1-2Manβ1-2Manα1-2Manα1-2Manα1-2Man. To assess the substrate specificity of β-1,2-ManTase II in the *C. albicans* serotype A cells, several PA-oligosaccharides prepared from mannan of *C. albicans*, *S. kluyveri*, and *P. pastoris* were tested. This result indicates that the enzyme requires not only the non-reducing terminal β-1,2-linked mannose unit, but also a penultimate α-1,2- or α-1,3-linked mannose unit as substrate (Table 1).[52] In other words, the minimum structural requirement of β-1,2-ManTase II is Manβ1-2Manα1-. Surprisingly, β-1,2-ManTase II activity was detected in the cells of *C. albicans* serotype B and *C. stellatoidea* strains, even though their mannan does not contain the serotype A-specific epitopes. To detect β-1,2-ManTase I in *C. albicans*, several α-1,2-linked mannooligosaccharide PA-derivatives were tested. However, no activity was detected. This result suggests that the enzyme recognizes the side chain length extending from the backbone α-1,6-linked mannose unit, and therefore requires the oligosaccharide-containing α-1,6-linked mannose unit as substrate. Although *C. albicans* mannan also contains α-1,2-linked mannobiose and mannotriose side chains, the β-1,2-linked mannose unit is transferred only to the α-1,2-linked mannotetraose side chain. On the other hand, in the case of *C. lusitaniae*[41] and *Citeromyces matritensis*[53] mannan,

β-1,2-linked mannose units are transferred only to the α-1,2-linked mannotriose and mannobiose side chains, respectively (Fig. 8). These structural differences in the species support the above hypothesis. On the other hand, *C. guilliermondii* mannan contains β-1,2-linked mannose units bound to α-1,3-linked mannose units.[43] Therefore, we tested the substrate specificity of β-1,2-ManTase I in *C. guilliermondii* cells. However, as shown in Table 1, linear oligosaccharides, which contain the non-reducing terminal α-1,3-linked mannose unit, do not serve as acceptors. Therefore, we enzymatically synthesized several 3,6-branch-containing oligosaccharides, Manα1-3(Manα1-6)Manα1-2Manα1-, by using α-1,6-ManTase of *C. albicans* serotype B cells with oligosaccharides from *C. albicans*, *C. krusei*, and *S. cerevisiae*. The results indicate that these 3,6-branched oligosaccharides are good acceptors.[54] We further performed large-scale enzymatic modification of the *C. albicans* serotype B mannan, which does not contain the β-1,2-linked mannose unit in its side chains, but the 3,6-branched structure, Manα1-3(Manα1-6)Manα1-. The incorporation of β-1,2-linked mannose units into mannan using the transferase of *C. guilliermondii* was confirmed by the appearance of β-1,2-linked mannose-specific cross-peaks in the TOCSY.

Mille *et al.*[55] cloned and characterized a novel β-1,2-ManTase gene family in *P. pastoris* and *C. albicans*. They first performed a BLAST search of a *P. pastoris* genome sequence, for ManTase, using the

Table 1.  Substrate specificity of the mannosyltransferase activity in the enzyme fraction from *Candida* species

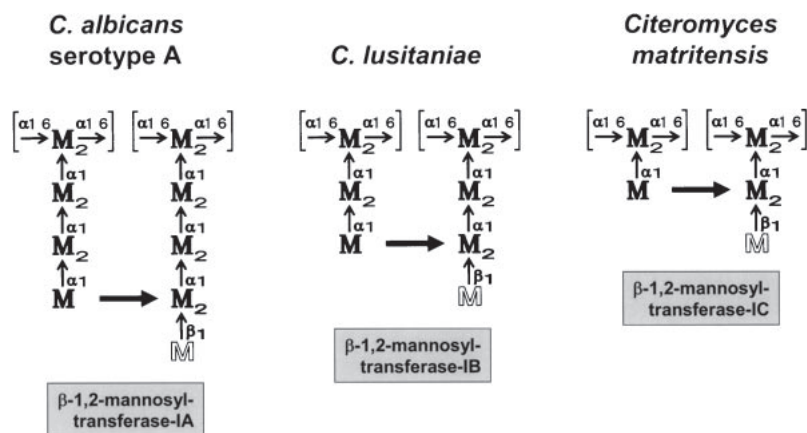| Substrate (5 mM) | Mannose incorporated (nmol/mg protein/h) | |
|---|---|---|
| | *C. albicans* serotype A | |
| | β-1,2-Mannosyltransferase | |
| Manβ1-2Man-PA | 0 | |
| Manβ1-2Manβ1-2Man-PA | 0 | |
| Manβ1-2Manα1-2Man-PA | 20 | |
| Manβ1-2Manβ1-2Manβ1-2Man-PA | 0 | |
| Manα1-2Manα1-2Manα1-2Man-PA | 0 | |
| Manβ1-2Manα1-2Manα1-2Manα1-2Man-PA | 29 | |
| Manβ1-2Manα1-3Manα1-2Manα1-2Man-PA | 29 | |
| Manβ1-2Manβ1-2Manα1-2Manα1-2Manα1-2Man-PA | 0 | |
| | *C. guilliermondii* | |
| | β-1,2-Mannosyltransferase | α-1,6-Mannosyltransferase |
| Manα1-3Manα1-2Man-PA | 13 | 149 |
| Manα1-2Manα1-2Manα1-2Man-PA | 0 | 0 |
| Manα1-3Manα1-2Manα1-2Man-PA | 1 | 41 |
| Manα1-3Manα1-2Man-PA<br>↑6<br>Manα1 | 52 | —[a)] |
| Manα1-3Manα1-2Manα1-2Manα1-2Man-PA | 1 | 49 |
| Manα1-3Manα1-3Manα1-2Manα1-2Man-PA | 6 | 97 |
| Manα1-3Manα1-2Manα1-2Man-PA<br>↑6<br>Manα1 | 52 | — |
| Manα1-3Manα1-2Manα1-2Manα1-2Man-PA<br>↑6<br>Manα1 | 60 | — |
| Manα1-3Manα1-3Manα1-2Manα1-2Man-PA<br>↑6<br>Manα1 | 48 | — |

*a*) Not determined.



Fig. 8.   Hypothetical substrates of β-1,2-mannosyltransferase I of three *Candida* species.
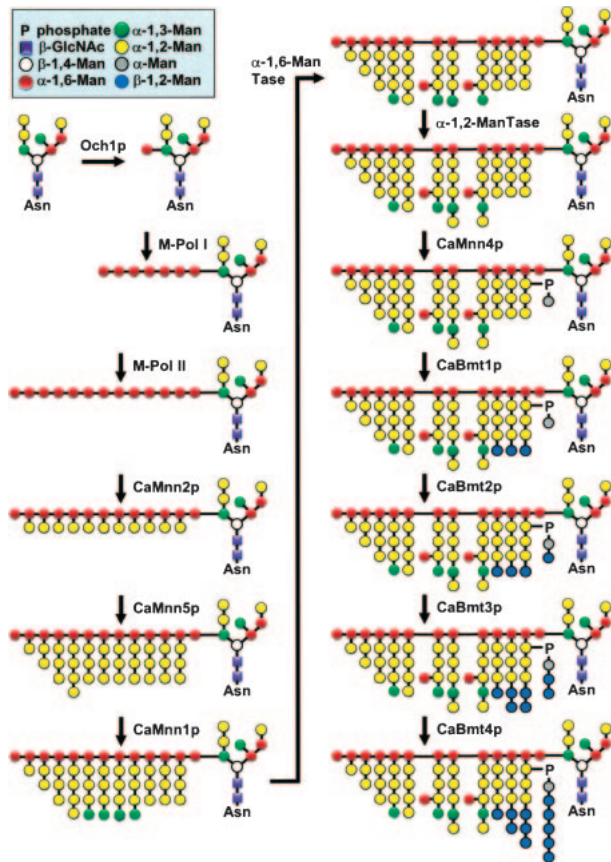
Fig. 9.   Biosynthesis pathway of N-linked mannan of *C. albicans*. The left side of the biosynthesis pathway is similar to that of *S. cerevisiae* and the right side is characteristic of *C. albicans*, the pathway of which involves the introduction of the α-1,6-branched mannose units and the β-1,2-linked mannose units in the side chains.

*S. cerevisiae* Mnn4p-containing lysine-glutamic acid rich repeats (KKKKEEEE) as a probe and found four new genes encoding β-1,2-ManTase. Using these sequence data, they subsequently identified nine homologs in *C. albicans*. They performed a deletion study of four genes, *CaBMT1-4*, and identified the reaction position of these β-1,2-ManTases in the biosynthesis pathway.[56] From the results of these structural and enzymatic analyses, the biosynthesis pathway of the mannan of *C. albicans* was deduced (Fig. 9).

## 8. Correlation between pathogenicity and mannan structure of *C. albicans* species

Adherence of *C. albicans* cells to host receptors is important for the establishment of colonization and initiation of invasion into host tissues. Therefore, the structure of the cell wall mannan has received

much attention. It was reported that the factor 6 antigen of *C. albicans* serotype A, which contains a β-1,2-linked mannose unit at the non-reducing terminal of the α-1,2-linked mannooligosaccharide, plays a role in fungal adherence to epithelial cells.[57] The factor 5 antigen, which corresponds to the β-1,2-linked mannooligosaccharide, has also been shown to induce tumor necrosis factor (TNF)-α and eicosanoid production through binding to macrophages via galectin-3.[58],[59] The α-linked oligomannosyl side chains have also been shown to have a strong adhesion activity.[60]–[62] With an inhibition assay using a monoclonal antibody specific for each synthetic oligosaccharide, Dalle *et al.*[63] demonstrated that β-1,2- and α-1,2-linked mannooligosaccharides participate in the adherence of *C. albicans* cells to enterocytes. The prevention of intestinal colonization by *C. albicans* through the oral administration of synthetic β-1,2-linked mannooligosaccharides was demonstrated by Dromer *et al.*[64] Moreover, Cutler and coworkers[65],[66] demonstrated that the monoclonal antibody against β-1,2-linked mannooligosaccharides protects against disseminated candidiasis and vaginal infection. Recently, Lee *et al.*[67] showed the effectiveness of a monoclonal antibody against β-1,2-linked mannooligosaccharides, in combination with fluconazole, against candidiasis.

Och1p, an α-1,6-ManTase, is responsible for the initiation of outer chain backbone synthesis. Bates *et al.*[68] showed that the virulence of an *och1Δ* null mutant of *C. albicans* was attenuated in a murine model of systemic infection. Bai *et al.*[69] also showed the importance of an α-1,2-ManTase responsible for the synthesis of the side chains of the N-linked mannan. They demonstrated that an *mnn5Δ* null mutant of *C. albicans* markedly reduced virulence.

Protein O-mannosyl transferases (Pmtp) participate in the first mannosylation step during the synthesis of O-linked mannooligosaccharides in the mannoprotein. Ernst and coworkers[70]–[72] showed that Pmtp(s) were required for growth, antifungal resistance, biofilm formation, hyphal formation, and virulence in *C. albicans*. The addition of further mannose units to the first O-linked mannose unit involves ManTases including Mnt1p and Mnt2p for the transfer of the second and third α-1,2-linked mannose units. Synthesis of the O-linked oligomannosyl moiety by Mnt1p and Mnt2p is required for adhesion of *C. albicans* cells to human buccal epithelial cells and to rat vaginal epithelial cells, and for virulence.[73],[74]
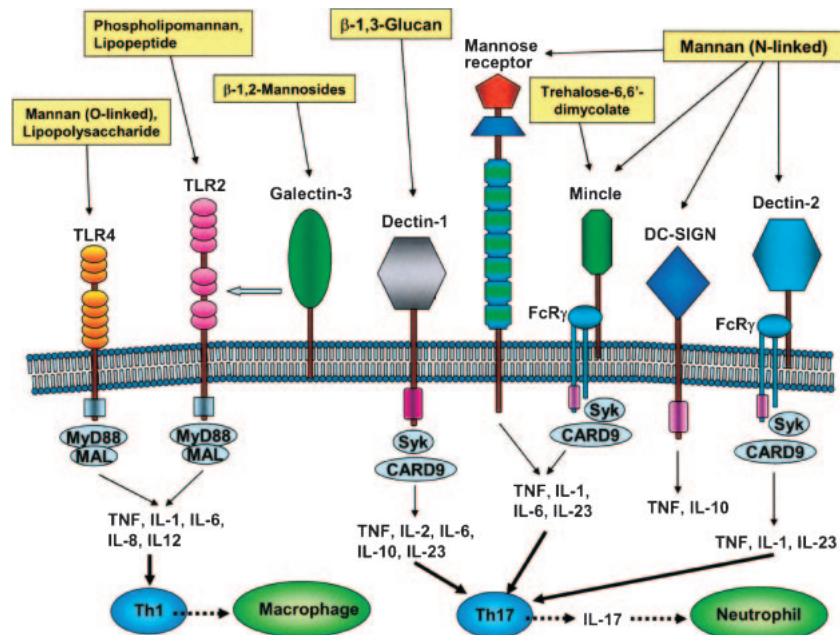
Fig. 10.   Pattern recognition receptors sensing *C. albicans*. The *C. albicans* mannan induces IL-1$\beta$ and IL-23 secretion in a Dectin-2-dependent manner and induces Th17 cell differentiation. IL-17A from Th17 cells recruits neutrophils to inflammatory sites.

## 9. Structural changes in *Candida* mannan under several growth conditions

*C. albicans* are known to be able to grow in two forms: as yeast cells and hyphal cells, a condition called dimorphism that is dependent on many factors such as growth temperature and the presence of serum or N-acetylglucosamine. We demonstrated a decrease in phosphate-bound $\beta$-1,2-linked manno-oligosaccharides in the mannan of hyphal cells compared to yeast cells.[25),35),38)] Growth of the cells under acidic conditions at pH 2.0[75),76)] or under high temperature conditions at 37°C[77),78)] instead of the standard growth temperature (25–28°C) causes a decrease or disappearance of $\beta$-1,2-linked mannose units in the mannan. Such a change in the epitopes *in vitro* can also be assumed to take place in patients. Since serological diagnosis for invasive candidiasis is directed at the detection of a specific circulating cell wall antigen or cytoplasmic antigen, the change in antigen profile will directly affect the sensitivity and specificity of diagnosis.

## 10. Phospholipomannan

Poulain and coworkers[79)–81)] found the existence of a glycolipid on the cell surface of *C. albicans*, the so-called phospholipomannan, composed of long linear $\beta$-1,2-linked mannooligosaccharides that reach up to 20 units in length, and phytoceramide with a spacer structure: -Man-P-Man-inositol-P-. They showed the involvement of the Toll-like receptor 2 (TLR2) in the phospholipomannan-induced TNF-$\alpha$ production by macrophages, which correlates with activation of the nuclear factor (NF)-$\kappa$B.[82)] Furthermore, they showed that the phospholipomannan promotes survival of phagocytosed *Candida* cells through activation of macrophage apoptosis by down-regulation of ERK1/2 signal transduction, a decrease in p90$^{rsk}$ and Bad phosphorylation, and elimination of free Bcl-2.[83)] Mille *et al.*[84)] showed that deletion of a ManTase responsible for the synthesis of phospholipomannan in *C. albicans* reduced virulence in mouse.

## 11. Recognition of *Candida* cells by innate immunity

C-type lectin receptors are a family of pattern-recognition receptors that recognize the structures of cell-surface polysaccharides. The mannose receptor, Dectin-2, dendritic-cell-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing non-integrin (DC-SIGN), and Langerin recognize $\alpha$-mannan and Dectin-1 recognizes $\beta$-1,3-glucan in the fungal cell wall. Some of these receptors are central to the fungal recognition and induction of the innate antifungal immune response. Dectin-2 recognizes high-mannose

structures[85),86)] and induces cytokine production by signaling through an Fc receptor $\gamma$ chain[85)] and in a Syk-CARD9-NF-$\kappa$B-dependent manner.[87)] Although the cytokine-inducing activity of the hyphal cell was much stronger than that of the yeast form, only Dectin-2 is responsible for the induction of cytokines, interleukin (IL)-1$\beta$, IL-6, IL-23, and TNF. Dectin-2 signaling by *Candida* mannan preferentially promotes Th17 cell differentiation, and IL-17A from Th17 cells recruits neutrophils to the inflammatory sites and activates both T and B cells (Fig. 10). DC-SIGN recognizes several endogenous ligands as well as microbial pathogens, including *C. albicans*, *Aspergillus fumigatus*, *Mycobacterium tuberculosis*, some viruses and protozoa via mannose and fucose moieties on the surface of the pathogens. Cambi *et al.*[88)] showed that recognition of the N-linked $\alpha$-mannan, but not the O-linked $\alpha$-mannooligosaccharides or phosphate-bound $\beta$-1,2-linked mannooligosaccharides, by DC-SIGN is important for binding and phagocytosis of *C. albicans* by human dendritic cells and production of the proinflammatory cytokine IL-6. Recently, the macrophage-inducible C-type lectin (Mincle) was also found to bind to mannan and *Candida* cells.[89),90)] Intelectin,[91)] a galactofuranose-binding lectin, also seems to participate in the recognition of many pathogenic fungi, such as *Aspergillus fumigatus*,[92)] *Trichophyton rubrum*,[93)] *Malassezia furfur*,[94)] and *Fonsecaea pedrosoi*,[95)] all of which have been found to contain galactofuranose units in their cell wall polysaccharides.

## 12. Conclusions

Epidemiological studies have revealed that emerging *Candida* species may vary geographically in frequency of isolation. Although the most common non-albicans in the United States is *C. glabrata*, in Latin America and Asia-Pacific region it is *C. parapsilosis* and *C. tropicalis*. It is also known that elevated rates of fluconazole resistance occur among the isolates of *C. glabrata*, *C. krusei*, *C. guilliermondii*, *C. rugosa*, and *C. famata*. Therefore, it is important to identify the clinical isolates of *Candida* at the species level and/or by susceptibility testing. The adhesion of *Candida* to epithelial cells facilitates colonization and can be regarded as the first step in the pathogenesis of *Candida* infection. In this process cell wall mannooligosaccharide side chains appear to be involved as well as *Candida* cell-surface hydrophobic proteins, integrin analogs, fimbrial adhesins and lectin-like adhesins with specificity for L-fucose or D-GlcNAc. The increase in invasive candidiasis requires further investigation in order to understand the mechanisms of pathogenesis, the findings of which may provide a new immunity-based therapy or prophylaxis.

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## Profile

Nobuyuki Shibata was born in 1956 in Tochigi, Japan. He received his Ph.D. in 1989 from Tohoku College of Pharmacy (presently, Tohoku Pharmaceutical University) under the supervision of Prof. Shigeo Suzuki. He became an Assistant Professor at Tohoku College of Pharmacy in 1981, and was a postdoctoral fellow at the University of Minnesota with Prof. Robert D. Nelson in 1989–1990. He was promoted to Associate Professor in 2000 and then to Professor in 2011 at Tohoku Pharmaceutical University. His research interests are in the fields of carbohydrate chemistry of the cell wall polysaccharide of pathogenic fungi and its immunochemistry. He was awarded the Pharmaceutical Society of Japan Tohoku Branch Award for Young Scientists in 1991.

## Profile

Hidemitsu Kobayashi was born in 1961 in Osaka, Japan. He started his research career in 1984 with studies on the structural characterization of antigenic polysaccharides obtained from pathogenic eucaryotic microbe, mainly genus *Candida*, after graduating from the Tohoku Pharmaceutical University, Sendai, Japan. He was able to give the doctor's degree of pharmacology according to the achievements of the structural study of *Candia* cell wall mannan in 1991. From 1992 to 1993, he was on the register in Institut Pasteur, Paris, France, as a postdoctoral fellow, and engaged in research of the genus *Aspergillus* circulating antigen in the human body by instruction of Dr. Jean-Paul Latge. Then he moved to the Department of Microbiology, Faculty of Pharmaceutical Science, Nagasaki International University, Sasebo, Japan, as a professor in 2004. He started to study genus *Cordyceps*, a group of fungi parasitic on viable insects in 2008. These microorganisms are called Toh-Chu-Kaso, which has been used as a drug for longevity in China. Recently, he discovered some low molecular compounds with strong antitumor activity from the Toh-Chu-Kaso, and the research is still continued.

## Profile

Shigeo Suzuki was born in Sendai at 1929. He graduated from Tohoku College of Pharmacy (presently, Tohoku Pharmaceutical University) in 1950. He started his carbohydrate research at 1951 in the Department of Hygienic and Forensic Chemistry, Faculty of Pharmaceutical Sciences at the University of Tokyo as a researcher. Prof. S. Akiya and Assoc. Prof. S. Okui (later, Prof. of Tohoku University) of this department were conducting a work, "Studies on the degradation process of sugars". He participated in the publication of 3 papers of this series at 1952. After publishing 2 carbohydrate-relating papers, Prof. Akiya suggested to start a research on sugar-amino acid conjugates. He returned to the Department of Hygienic Chemistry, Tohoku College of Pharmacy at 1958, and this work was continued Prof. T. Ukita, the successor of Prof. Akiya, as co-author. At 1961, He was awarded with Ph.D. degree of the University of Tokyo. In 1965, He elevated to the professor of Second Department of Hygienic Chemistry, and started a series of immunochemical investigation of *Candida* mannan as described in the text. He was honored with the following prizes: 1. The Japanese Society for Medical Mycology Prize at 1992. 2. The International Society for Human and Animal Mycology Prize at 1997. 3. Naito Memorial Scientific Research Foundation Grant at 1997. He is the honorary members of the following societies: 1. The Japanese Society for Carbohydrate Research. 2. The Japanese Society of Medical Mycology. 3. The Japanese Society for Chitin·Chitosan Research.