

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.801.311>

Distribution of Oligomannosyl Side Chains in the Cell Wall Mannan of *Pichia pastoris* Purified by Benanomicin A

Takuya Kuraoka¹, Momoka Shukuri¹, Saki Iwanaga¹, Takayoshi Yamada²,
Yukiko Ogawa¹ and Hidemitsu Kobayashi^{1*}

¹Laboratory of Microbiology, Department of Pharmacy, Faculty of Pharmaceutical Science,
Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo,
Nagasaki 859-3298, Japan

²Sanko Medical Tomato Pharmacy Co., Ltd., 2023-2, Shimogumigo, Higashisonogigun,
Kawatanacho, Nagasaki, 859-3615, Japan

*Corresponding author

ABSTRACT

In the previous study, a new method was established to prepare mannan under mild conditions by using antibiotic Benanomicin A. In this study, this method was applied to the isolation of *P. pastoris* NBRC 0984 mannan which predominantly contains β -1,2-linked mannose residues. As the findings of nuclear magnetic resonance (NMR) analysis of the resultant mannan to examine the distribution state of the side chain, it was found that despite the existence of oligomannosyl side chains corresponding to pentaose, $\text{Man}\alpha 1 \rightarrow 2\text{Man}\beta 1 \rightarrow \text{Man}\beta 1 \rightarrow \text{Man}\alpha 1 \rightarrow 2\text{Man}$, tetraose, $\text{Man}\beta 1 \rightarrow \text{Man}\beta 1 \rightarrow \text{Man}\alpha 1 \rightarrow 2\text{Man}$, and biose, $\text{Man}\alpha 1 \rightarrow 2\text{Man}$, in this molecule, the side chain corresponding to triose, $\text{Man}\beta 1 \rightarrow \text{Man}\alpha 1 \rightarrow 2\text{Man}$, was not detected at all. In our previous study, a relatively large number of biosyl and triosyl side chains were detected in analyzes applying acetolysis to mannans prepared by the Fehling method from the same yeast cells. Such a difference can be explained as that the β -1,2-linkages of some pentaosyl and tetraosyl side chains were cleaved by acetolysis, and triose and biose occurred secondarily in large quantities. In conclusion, the best way to accurately measure the side chain distribution of mannan is to perform NMR analysis on untreated mannan molecules prepared under mild conditions using Benanomicin A.

Keywords

Pichia pastoris,
Cell wall mannan,
Benanomicin A,
Nuclear Magnetic
Resonance (NMR)

Article Info

Accepted:
20 December 2018
Available Online:
10 January 2019

Introduction

In carbohydrate biochemistry, acetolysis is the one of the important procedures for the selective cleavage of α -1,6-linkages. This method was frequently used for the structural and immunochemical studies of various yeast mannans (Kocourek *et al.*, 1969; Shibata *et*

al., 2012), and for the preparation of several substrates for transferases in biosynthetic studies of yeast mannan (Suzuki *et al.*, 1996; Shibata and Okawa, 2010). Gorin and Perlin (Gorin and Perlin, 1956) first applied acetolysis to the structural study of yeast mannans, and were succeed to isolate $\text{Man}\alpha 1 \rightarrow 2\text{Man}$. Thereafter, many reports

were published utilizing acetolysis to structural study of various yeast mannans (Kocourek *et al.*, 1969; Suzuki and Sunayama, 1968 a, b; Gorin *et al.*, 1969; Hamada *et al.*, 1981; Funayama *et al.*, 1984). At about the same time, Gorin and Spencer (Gorin and Spencer, 1970) indicates that some yeast mannans contain β -linked mannose residues from the results of NMR studies. After a while, Kobayashi *et al.* (Kobayashi *et al.*, 1986, 1988) developed mild acetolysis that be able to isolate manno oligosaccharides containing α -1,2- and β -1,2-linkages from the mannan of *Pichia pastoris*. After that, this method was widely used the analysis for chemical structure of cell wall mannan of pathogenic yeast, genus *Candida* (Kobayashi *et al.*, 1987, 1989, 1992 a). At the same time as these studies, NMR development and dissemination were achieved, and this technical application enabled more detailed analysis of mannan sugar chains (Kobayashi *et al.*, 1990; Shibata *et al.*, 1993 a).

In carrying out structural analysis of yeast mannan, one of most important point is separating and refining of intact mannan molecule. What used to isolate yeast cell wall mannan so far is a method of precipitating mannan as a copper complex using a Fehling reagent. However, since this preparation method has a step of immersing in a strongly alkaline solution, there is a possibility that the resultant mannan has been damaged. Therefore, in the previous study (Kuraoka *et al.*, 2018), we developed a new mannan preparation method using Benanomicin A which is an antibiotic having lectin-like activity. In this way, the mannan composed only of α -linked mannose residues could be prepared successfully from pathogenic yeast *Candida krusei* (Kuraoka *et al.*, 2018).

In the present study, we demonstrate the usefulness of new purification method of yeast cell wall mannan using Benanomicin A.

Namely, we adopted a new method for the preparation of intact mannan containing a large number of β -1,2-linkage from *P. pastoris* NBRC 0984 (formerly IFO 0984). Then, this mannan was analyzed by NMR, two-dimensional isotope Hartmann-Hearn (2D-HOHAHA) and ^{13}C - ^1H correlation spectroscopy (C-H COSY) to ascertain the exact distribution of oligomannosyl side chains.

Materials and Methods

General

Pichia pastoris NBRC 0948 (formerly IFO 0948) strain were obtained from the Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Japan. The cells were cultivated in the yeast extract-Sabouraud's liquid medium [0.5% (w/v) yeast extract, 1% (w/v) peptone, and 2% (w/v) glucose] at 27°C for 72 hours on a reciprocal shaker.

Benanomicin A was kindly provided by Dr. Shuichi Gomi (Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd.).

Preparation of crude extract from *Pichia pastoris* NBRC 0948

This was prepared according to the previous report (Kuraoka *et al.*, 2018). Yield of crude extract was 13.2% based on an acetone-dried cells weight.

Preparation of cell wall mannans of *P. pastoris* by two different procedures

Preparation of mannan using Benanomicin A was performed according to previously described (Kuraoka *et al.*, 2018). This method is referred to as a Benanomicin method. Purified mannan by this method is abbreviated as Fr. P-B. On the other hand, mannan was

prepared using Fehling reagent as previously described (Okubo *et al.*, 1978). Fehling reagent consists of a 1:1 (v/v) mixture of 3.5% CuSO₄ 5H₂O, 17.3% Rochelle salt and 5.0% NaOH. This method is referred to as the Fehling method. Purified mannan by this method is abbreviated as Fr. P-F.

NMR analysis of mannans

¹H-NMR spectra (internal acetone, 2.217 ppm) were measured with a Jeol JNM-GSX 400 spectrometer on solutions (3-10mg sample/0.7mL) in D₂O at 70°C. ¹³C-NMR spectra (internal CD₃OD, 49.00 ppm) were measured with the same spectrometer on solutions (15-25mg sample/0.7mL) in D₂O at 55°C. 2D-HOHAHA spectrum was recorded for a solution (10 mg sample/0.7mL) in D₂O at 45°C. ¹³C-¹H COSY spectra were also recorded under the same conditions as for the ¹H and ¹³C NMR spectra. The percentage of the different kinds of oligomannosyl side chains in mannan was calculated based on the peak-dimensions of the corresponding H-1 signals in the ¹H-NMR spectrum (Kobayashi *et al.*, 1997).

Calculation of average length of side chains

The average length of side chains (X) was calculated by using the following formula in accordance with previous descriptions (Kobayashi *et al.*, 2003):

$$X = [(A \times 1) + (B \times 2) + (C \times 3) + (D \times 4) + (E \times 5)] / (A + B + C + D + E),$$

Where A through E represent the molar proportions of mannose, biose, triose, tetraose, and pentaose in the peak-dimensions of the corresponding H-1 signals in the ¹H-NMR spectrum, and the numbers 1 through 5 indicate the degrees of polymerization of the mannose (M₁) and the four oligosaccharides, M₂ through M₅, respectively.

Results and Discussion

¹H-NMR analysis of mannans, Fr. P-B and P-F

The chemical structure of mannan was analyzed by means of ¹H-NMR (Figure 1). The spectra of both fractions showed extremely similar patterns in the anomeric proton region (range of 4.7 to 5.7 ppm). This finding supports the new purification method of yeast mannan being as effective as conventional methods. Therefore, it is cleared that Benanomicin method was successfully prepared even mannan having a large amount of β-linkage. The absence of any signal in the range of 5.40 to 5.70 ppm indicates that both fractions do not contain mannose residues via a phosphodiester bond. Three strong signals derived from α-1,2- and α-1,6-linked mannose residues (5.100, 5.071 and 4.876 ppm), and four strong signal derived from α-1,2 and β-1,2-linked mannose residue (5.376, 5.159, 4.844 and 4.834 ppm) were observed in the pattern of both fractions. In summary, as a result of assignment of each signal based on our previous report (Gorin *et al.*, 1969), it became clear that cell wall mannan contains α-1,2-, α-1,6- and β-1,2-linked mannose residue. These results are consistent with previous report (Kobayashi *et al.*, 1986). Therefore, we conducted following experiments using Fr. P-B.

Two-dimensional NMR analysis of Fr. P-B

Verification of overall structure of Fr. P-B was attempted by chemical shift values of cross-peaks on two kinds of two-dimensional NMR maps. Figure 2 shows the two-dimensional map of 2D-HOHAHA spectrum of Fr. P-B. Nine cross-peaks, identified based on previous reports (Shibata *et al.*, 1993 a, 1996), were observed. Namely, the appearance of cross-peaks 1, 2 and 3 indicates that mannan contain β-1,2-linked oligomannosyl side chains.

Cross-peaks 6, 7 or 4 correspond to the 2-*O*-substituted or unsubstituted forms of the α -1,6-linked polymannosyl backbone, respectively. On the other hand, the presence of α -1,2-linked mannose residues was demonstrated by the appearance of cross-peaks 5 and 8. Cross-peak 9 indicates the existence of α -1,2-linked mannose residues in the non-reducing terminal of long side chain. Figure 3 shows the two-dimensional map of ^{13}C - ^1H COSY spectrum of Fr. P-B. Eleven cross-peaks, identified based on previous reports (Shibata *et al.*, 1993 b), were observed. The presence of cross-peak C, D, I and J indicates that mannan contain β -1,2-linked oligomannosyl side chains. Cross-peaks A, B or E correspond to the 2-*O*-substituted or unsubstituted forms of the backbone in which α -1,2-linked mannose residues are polymerized, respectively. The existence of α -1,2-linked mannose residues was confirmed by the appearance of cross-peaks F, H and K. Cross-peak G indicates the existence of α -1,2-linked mannose residues in the non-reducing terminal of long side chain. Therefore, the chemical shifts in the spectra of Fr. P-B were assigned according to published data (Shibata *et al.*, 1993 b, 2010; Kobayashi *et al.*, 1991, 1992 b, 1998; Shibata *et al.*, 2007; Oyamada

et al., 2008), as in Table 1. It is cleared that this mannan has oligomannosyl side chain corresponding to biose ($\text{Man}\alpha 1 \rightarrow 2\text{Man}$), tetraose ($\text{Man}\beta 1 \rightarrow 2\text{Man}\beta 1 \rightarrow 2\text{Man}\alpha 1 \rightarrow 2\text{Man}$) and pentaose ($\text{Man}\alpha 1 \rightarrow 2\text{Man}\beta 1 \rightarrow 2\text{Man}\beta 1 \rightarrow 2\text{Man}\alpha 1 \rightarrow 2\text{Man}$).

Overall structure of Fr. P-B

As shown in Figure 4, the distribution of the side chains in Fr. P-B was calculated based on the dimensions of the characteristic H1 signals of the side chains labeled with asterisks in Table 1. The distribution of side chains of *P. pastoris* mannans which were analyzed by two kinds of procedures was clearly different. Particularly remarkable is that NMR analysis of Fr. P-B did not detect a triosyl side chain (Figure 4A). In the previous paper (Kobayashi *et al.*, 1986), the triose, $\text{Man}\beta 1 \rightarrow \text{Man}\alpha 1 \rightarrow 2\text{Man}$, as acetolysate has been obtained from mannan prepared by Fehling method (Figure 4B: the part surrounded by the dotted line). In addition, the distribution of side chains of Fr. P-B was calculated from the dimensions of anomeric proton (H-1) signals in the NMR spectrum.

Figure.1 ^1H -NMR spectra (anomeric region) of *P. pastoris* mannan, Frs. P-B (A) and P-F (B)

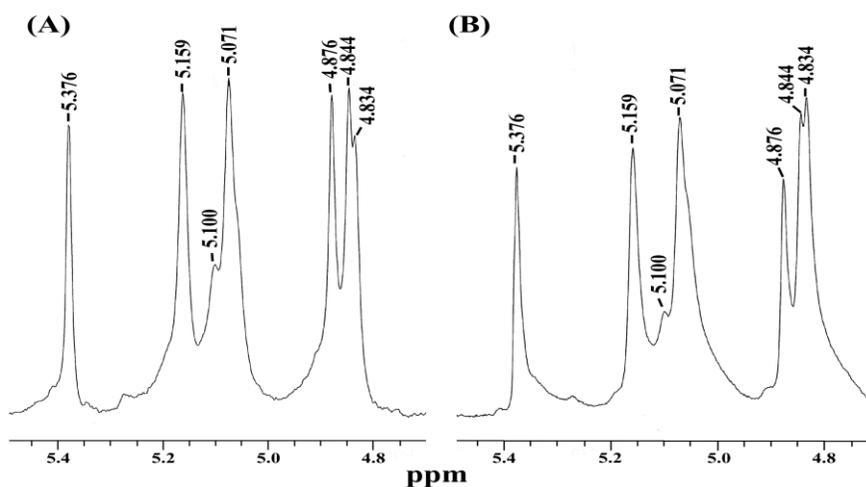


Table.1 Assignment of ¹H and ¹³C NMR chemical shifts of Fr. P-B. Man indicate mannose residue. Side chain sequence is not specified. Asterisks indicate characteristic H1 signal of each side chain

	Mannose residue			
	→ 6Manα1	→ 6Manα1 ↑ 2 Manα1	→ 6Manα1 ↑ 2 Manα1 ↑ 2 Manβ1 ↑ 2 Manβ1	→ 6Manα1 ↑ 2 Manα1 ↑ 2 Manβ1 ↑ 2 Manβ1 ↑ 2 Manα1
	Chemical shift (ppm)			
H-1	4.91*	5.09	5.06	5.06
H-2	4.00	4.03	4.07	4.07
C-1	100.33	99.24	99.57	99.57
H-1		5.05*	5.15	5.15
H-2		4.08	4.26	4.26
C-1		102.99	100.78	100.84
H-1			4.83	4.82
H-2			4.25	4.06
C-1			99.79	100.19
H-1			4.84*	4.87
H-2			4.25	4.17
C-1			101.82	101.57
H-1				5.37*
H-2				4.07
C-1				100.97

Figure.2 2D-HOHAHA spectrum of Fr. P-B. Boxed cross-peaks are H-1 and H-2 correlated by J-coupling

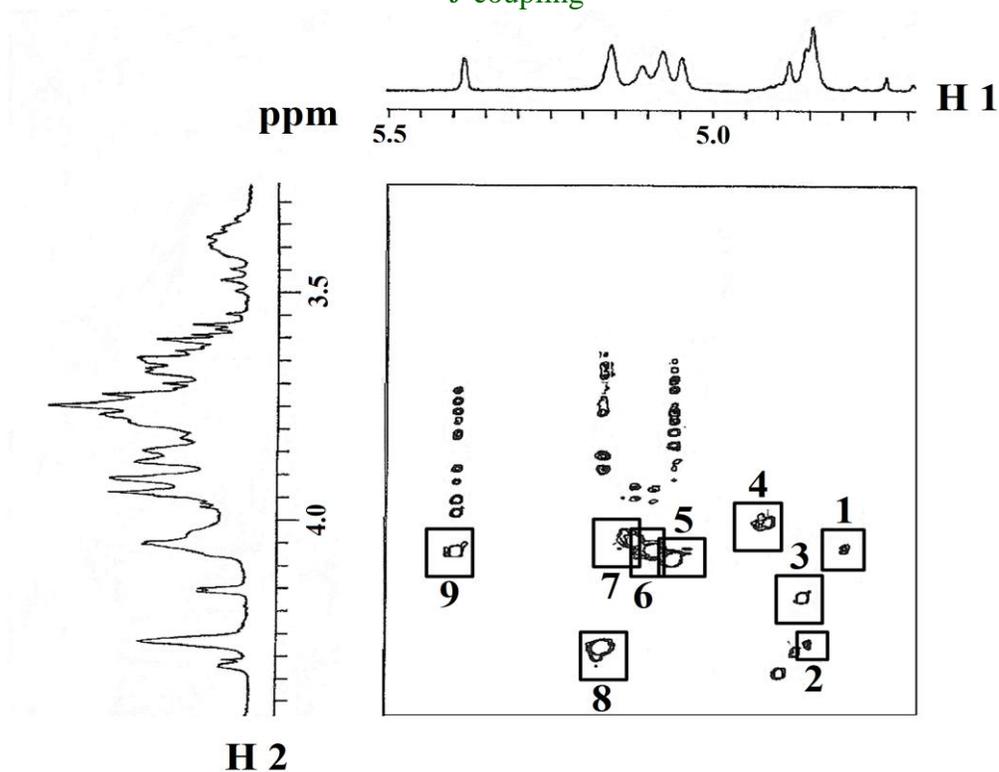


Figure.3 ¹³C-¹H COSY spectrum of Fr. P-B. Boxed cross-peaks are H-1 and C-1 correlated by J-coupling

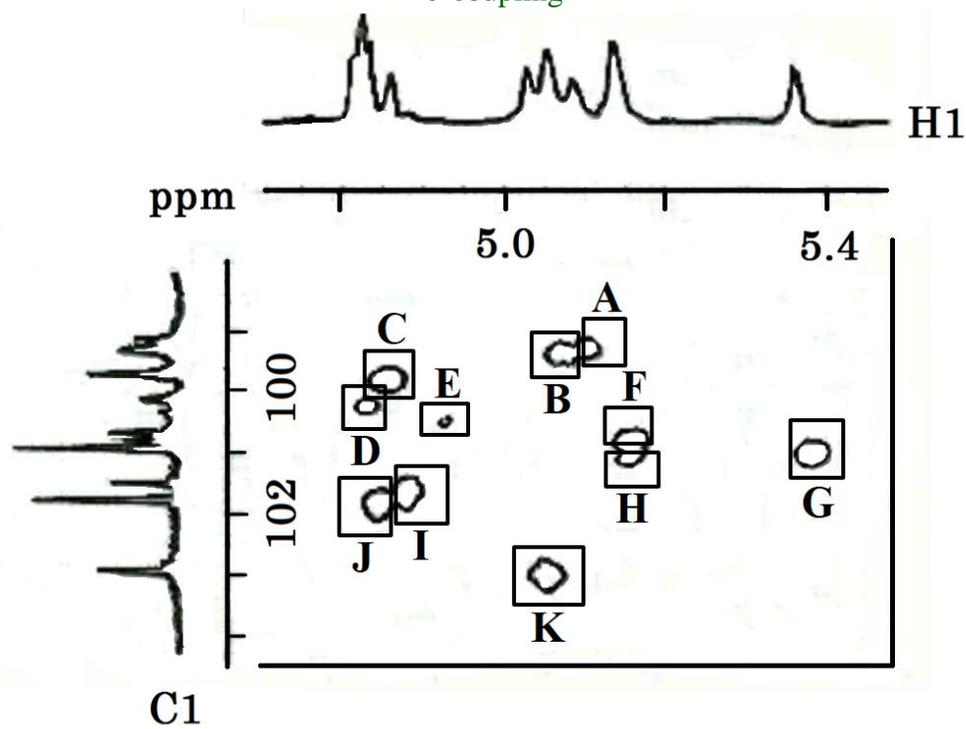
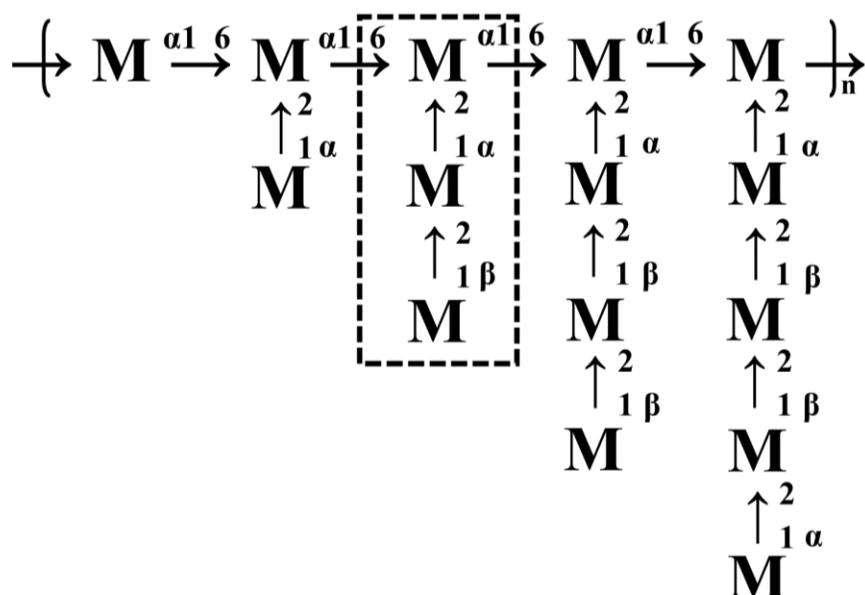


Figure.4 Structure of *P. pastoris* NRBC 0948 mannan. (A) Side chain distribution was calculated based on the dimensions of characteristic H-1 signals of each side chain in the ¹H-NMR spectroscopy map (Shibata *et al.*, 1996). (B) Side chain distribution was calculated based on the peak-dimensions in the gel-filtration profile of the mild acetolysis products. M indicate mannose residue. *These values were referred from previous report (Kobayashi *et al.*, 1988). Side chain sequence is not specified. The part surrounded by the dotted line is indicates that it was not detected by NMR



Side chain distribution

(A)	9.60	:	26.36	:	-	:	36.85	:	27.19
(B)*	14.64	:	34.47	:	4.06	:	32.32	:	14.51

Total molar ratio of tetraosyl and pentaosyl side chains, 64.04 (Figure 4A), was higher than that of same oligonosyl side chain calculated by elution profile of acetolysates in our previous description (Kobayashi *et al.*, 1986), 46.83 (Figure 4B). Conversely, the total ratio of biosyl side chains and non-substituted backbone moieties of Fr. P-B, 35.96, was calculated to be much lower than the same ratio in our previous report (Kobayashi *et al.*, 1986), 49.11. Comparing the average lengths of the side chains of two mannans analyzed by different procedures, it was 3.5 for Fr. P-B, whereas the mannan in

the previous report (Kobayashi *et al.*, 1986) was relatively short, 3.0.

In this study, we demonstrated that the new yeast mannan preparation method using Benanomicin A developed in our previous report (Kuraoka *et al.*, 2018) is applicable not only to mannan composed of α -linkage but also to preparation of mannan containing β -linkage. Therefore, it is of interest whether the antibiotic Benanomicin A can also be used for preparing heteropolysaccharides containing mannose residues.

The fact that the distribution of side chains of *P. pastoris* mannans analyzed by the two procedures is clearly different suggests that a part of a slightly longer side chain of mannan is hydrolyzed in the process of acetolysis. This interpretation is also supported by the difference in the average chain length of both mannans. Additionally, no detection of the triosyl side chains in the *P. pastoris* mannan suggests that the β -1,2-mannosyltransferases work more quickly than α -1,2-mannosyltransferases and contribute to the construction of tetraosyl and pentaosyl side chains in the biosynthetic process of mannan.

In conclusion, it is certain that polysaccharide resolution, such as acetolysis, is useful for preparing oligosaccharides corresponding to key fragments of various immunochemical or biological functions. However, as a tool for obtaining accurate information on the chemical structure of an intact polysaccharide, various applied NMR analyzes are optimal rather than restricted decomposition methods. Therefore, it was shown in this report, one of the most convenient structural analyzes of yeast mannan is a method using Benanomycin A and two-dimensional NMR.

References

- Funayama, M., Nishikawa, A., Shinoda, T., Suzuki, M., Fukazawa, Y., 1984. Antigenic relationship between *Candida parapsilosis* and *Candida albicans* serotype B. *Microbiol. Immunol.* 28, 1359-1371.
- Gorin, P.A.J., Perlin, A.S., 1956. A mannan produced by *Saccharomyces rouxii*. *Canad. J. Chem.* 34, 1796-1803.
- Gorin, P.A.J., Spencer, J.F.T., 1970. Proton magnetic resonance spectroscopy-an aid in identification and chemotaxonomy of yeasts. *Adv. Appl. Microbiol.* 13, 25-89.
- Gorin, P.A.J., Spencer, J.F.T., Bhattacharjee, S.S., 1969. Structures of yeast mannans containing both α - and β -linked D-mannopyranose units. *Can. J. Chem.* 47, 1499-1505.
- Hamada, T., Nakajima, T., Izaki, K., Matsuda, K., 1981. Comparison of the mannan structure from the cell wall mutant *Candida* sp. M-7002 and its wild type. *Eur. J. Biochem.* 119, 365-371.
- Kobayashi H, Shibata N, Watanabe M, Komido M, Hashimoto, N., Hisamichi, K., Suzuki, S., 1992 b. Mild acetolysis and NMR studies of the D-mannan of *Saccharomyces cerevisiae* X2180-1A wild-type strain. *Carbohydr. Res.* 231, 317-323.
- Kobayashi, H., Giummelly, P., Takahashi, S., Ishida, M., Sato, J., Takaku, M., Nishidate, Y., Shibata, N., Okawa, Y., Suzuki, S., 1991. *Candida albicans* serotype A strains grow in yeast extract-added Sabouraud liquid medium at pH 2.0, elaborating mannans without β -1,2 linkage and phosphate group. *Biochem. Biophys. Res. Commun.* 175, 1003-1009.
- Kobayashi, H., Mitobe, H., Takahashi, K., Yamamoto, T., Shibata, N., Suzuki S., 1992 a. Structural study of a cell wall mannan-protein complex of the pathogenic yeast *Candida glabrata* IFO 0622 strain. *Arch. Biochem. Biophys.* 294, 662-9.
- Kobayashi, H., Oyamada, H., Iwadate, N., Szuki, H., Mitobe, H., Takahashi, K., Shibata, N., Suzuki, S., Okawa, Y., 1998. Structural and immunochemical characterization of β -1,2-linked mannobiosyl phosphate residue in the cell wall mannan of *Candida glabrata*. *Arch. Microbiol.* 169, 188-194.
- Kobayashi, H., Oyamada, H., Matsuda, K., Shibata, N., Suzuki, S., 2003. Distribution of antigenic

- oligomannosyl side chains in the cell wall mannans of several strains of *Candida tropicalis*. *Arch. Microbiol.* 180, 76-80.
- Kobayashi, H., Shibata, N., Mitobe, H., Ohkubo, Y., Suzuki, S., 1989. Structural study of phosphomannan of yeast-form cells of *Candida albicans* J-1012 strain with special reference to application of mild acetolysis. *Arch. Biochem. Biophys.* 272, 364-375.
- Kobayashi, H., Shibata, N., Nakada, M., Chaki, S., Mizugami, K., Ohkubo, Y., Suzuki, S., 1990. Structural study of cell wall phosphomannan of *Candida albicans* NIH B-792 (serotype B) strain, with special reference to ^1H and ^{13}C NMR analyses of acid-labile oligomannosyl residues. *Arch. Biochem. Biophys.* 278, 195-204.
- Kobayashi, H., Shibata, N., Suzuki, S., 1986. Acetolysis of *Pichia pastoris* IFO 0948 strain mannan containing α -1,2 and β -1,2 linkages using acetolysis medium of low sulfuric acid concentration. *Arch. Biochem. Biophys.* 245, 494-503.
- Kobayashi, H., Shibata, N., Suzuki, S., 1987. Structural study of phosphomannan-protein complex of *Citeromyces matriensis* containing β -1,2 linkage. Application of partial acid degradation and acetolysis techniques under mild conditions. *Arch. Biochem. Biophys.* 256, 381-396.
- Kobayashi, H., Shibata, N., Suzuki, S., 1988. Application of mild acetolysis to confirm the comb-like structure of cell wall mannan from *Pichia pastoris* IFO 0948 strain. *Chem. Pharm. Bull.* 36, 3168-3172.
- Kobayashi, H., Suzuki, J., Tanaka, S., Kiuchi, Y., Oyamada, H., Iwadate, N., Suzuki, H., Shibata, N., Suzuki, S., Okawa, Y., 1997. Structure of a cell wall mannan from the pathogenic yeast, *Candida catenulata*: Assignment of ^1H nuclear magnetic resonance chemical shifts of the inner α -1,6-linked mannose residues substituted by a side chain. *Arch. Biochem. Biophys.* 341, 70-74.
- Kocourek, J., Ballou, C.E., 1969. Method for fingerprinting yeast cell wall mannans. *J. Bacteriol.* 100, 1175-1181.
- Kuraoka, T., Ishiyama, A., Oyamada, H., Ogawa, Y., Kobayashi, H., 2018. Presence of O-glycosidically linked-oligosaccharides in the cell wall mannoprotein of *Candida krusei* purified with Benanomycin A. "in press"
- Okubo, Y., Ichikawa, T., Suzuki, S., 1978. Relationship between phosphate content and immunochemical properties of subfractions of bakers' yeast mannan. *J. Bacteriol.* 136, 63-68.
- Oyamada, H., Ogawa, Y., Shibata, N., Okawa, Y., Suzuki, S., 2008. Structural analysis of cell wall mannan of *Candida sojae*, a new yeast species isolated from defatted soybean flakes. *Arch. Microbiol.* 189, 483-890.
- Shibata, N., Akagi, R., Hosoya, T., Kawahara, K., Suzuki, A., Ikuta, K., Kobayashi, H., Hisamichi, K., Okawa, Y., Suzuki, S., 1996. Existence of novel branched side chains containing β -1,2 and α -1,6 linkages corresponding to antigenic factor 9 in the mannan of *Candida guilliermondii*. *J. Biol. Chem.* 271, 9259-9266.
- Shibata, N., Hisamichi, K., Kobayashi, H., Suzuki, S., 1993 b. Complete assignment of ^1H and ^{13}C nuclear magnetic resonance chemical shifts of β -1,2-linked manno-oligosaccharides isolated from the phosphomannan of the pathogenic yeast *Candida albicans* NIH B-792 strain. *Arch. Biochem. Biophys.* 169, 188-94.
- Shibata, N., Kobayashi, H., Suzuki, S., 2012.

- Immunochemistry of pathogenic yeast, *Candida* species, focusing on mannan. *Proc. Jpn. Acad. Ser. B. Phys. Biol. Sci.* 88, 250-265.
- Shibata, N., Kojima, C., Satoh, Y., Satoh, R., Suzuki, A., Kobayashi, H., Suzuki, S., 1993 a. Structural study of a cell-wall mannan of *Saccharomyces kluyveri* IFO 1685 strain. Presence of a branched side chain and β -1,2-linkage. *Eur. J. Biochem.* 217, 1-12.
- Shibata, N., Okawa, Y., 2010. Enzymatic synthesis of new oligosaccharides using mannosyltransferases from *Candida* species and their NMR assignments. *Biol. Pharm. Bull.* 33, 895-899.
- Shibata, N., Suzuki, A., Kobayashi, H., Okawa, Y., 2007. Chemical structure of the cell-wall mannan of *Candida albicans* serotype A and its difference in yeast and hyphal forms. *Biochem. J.* 404, 365-372.
- Suzuki A, Shibata N, Suzuki M, Saitoh F, Takata Y *et al.* (1996) Characterization of α -1,6-mannosyltransferase responsible for the synthesis of branched side chains in *Candida albicans* mannan. *Eur J Biochem* 240: 37-44.
- Suzuki, S., Sunayama, H., 1968 b. Studies on the antigenic activities of yeasts. II. Isolation and inhibition assay of the oligosaccharides from acetolysate of the mannan of *Candida albicans*. *Jpn. J. Microbiol.* 12, 413-422.
- Suzuki, S., Sunayama, H., Saito, S., 1968 a. Studies on the antigenic activity of yeasts. I. Analysis of the determinant groups of the mannan of *Saccharomyces cerevisiae*. *Jpn. J. Microbiol.* 12, 19-24.

How to cite this article:

Takuya Kuraoka, Momoka Shukuri, Saki Iwanaga, Takayoshi Yamada, Yukiko Ogawa and Hidemitsu Kobayashi. 2019. Distribution of Oligomannosyl Side Chains in the Cell Wall Mannan of *Pichia pastoris* Purified by Benanomycin A. *Int.J.Curr.Microbiol.App.Sci.* 8(01): 2926-2935. doi: <https://doi.org/10.20546/ijcmas.2019.801.311>