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Preparation and Analysis of Yeast Cell Wall Mannoproteins, Immune Enhancing Materials, from Cell Wall Mutant *Saccharomyces cerevisiae*

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Abstract Yeast cell wall matrix particles are composed entirely of mannoprotein and β-glucan. The mannoproteins of yeast cell wall can systemically enhance the immune system. We previously purified and analyzed alkali-soluble β-glucans [β-(1,3)- and β-(1,6)-glucans] [10]. In the present study, a wild-type strain was first mutagenized with ultraviolet light, and the cell wall mutants were then selected by treatment with 1.0 mg/ml laminarinase (endo-β-(1,3)-D-glucanase). Mannoproteins of *Saccharomyces cerevisiae* were released by laminarinase, purified by concanavalin-A affinity and ion-exchange chromatography. The results indicated that the mutants yielded 3-fold more mannoprotein than the wild-type. The mannoprotein mass of mutant K48L3 was 2.25 mg/100 mg of yeast cell dry mass. Carbohydrate analysis revealed that they contained mannose, glucose, and N-acetylglucosamine. *Saccharomyces cerevisiae* cell wall components, mannoproteins, are known to interact with macrophages through receptors, thereby inducing release of tumor necrosis factor alpha (TNF-α) and nitric oxide. Mannoprotein fractions in the present study had a higher macrophage activity of secretion of TNF-α and nitric oxide and direct phagocytosis than positive control (1 μg of lipopolysaccharide). In particular, F1 and F3 fractions in mannoproteins of K48L3 enhanced and upregulated the activity of nitric oxide secretion and macrophage phagocytosis by approximately two- and four-fold, respectively.

Key words: Mannoprotein, yeast cell wall, random mutation, immune activity

In the yeast *Saccharomyces cerevisiae,* the cell wall contains β-(1,3)-D-glucan, β-(1,6)-D-glucan, chitin, and mannoproteins. Polysaccharides appear to have a structural function, whereas mannoproteins may be "filler" and are important for the permeability of the cell wall. All four structural components of the cell wall, β-(1,3)-glucan, β- (1,6)-glucan, chitin, and mannoproteins, are linked together, and most of the proteins are covalently linked to the mannan, which is more correctly described as mannoproteins. The proportions of these different components vary with the species, but there are approximately equal proportions of mannan and glucan in *S. cerevisiae* [9].

The cell wall mannoproteins are usually of high molecular weight, carrying large amounts of N- and/or O-linked mannose polysaccharides [1]. Mannoprotein, with a protein moiety about 100 kDa in apparent size, is attached to β-(1,6)-glucan through a remnant of a glycosylphosphatidylinositol anchor containing five α-linked mannosyl residues. The basis of cell wall association of mannoproteins is not well understood. Some mannoproteins can be extracted from the cell wall with detergent, whereas others are covalently bound and can be released with lyticase [4, 22, 23]. Proteins in the latter part have common features, including a region rich in serine and threonine, and a glycosylphosphatidylinositol (GPI) anchor attachment signal at C-termini [2, 4]. Some cell wall mannoproteins have covalently associated β-(1,6)-glucan. The SDS-extractable mannoproteins appear to be noncovalently associated with the cell wall. Lyticase-extractable mannoproteins have been shown to contain glucose residues, and it has been proposed that this class of mannoproteins is cross-linked to the wall β-glucan [10, 31, 32]. The yeast cell wall mannoproteins act as nonspecific modulators of the immune system. The biological activities of the mannoproteins include host-mediated antitumor activity [18], adjuvant effects [19], and activation of

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neutrophils, eosinophils, macrophages, and complement [5, 33].

In the present study, we mutated the cell wall of *Saccharomyces cerevisiae*. Mannoproteins were separated from the cell wall and analyzed by various chromatographies. Furthermore, we investigated the capacity of mannoproteins to stimulate the human immune system by quantifying phagocytosis and by measuring TNF-β and nitric oxide.

MATERIALS AND METHODS

Strains and Cultivation

Saccharomyces cerevisiae YPH499 (Mat a, ade2-101, his3Δ200, leu2, lys2-801, ura3-52, trp1-Δ1) was used for ultraviolet (UV)-induced mutagenesis. *S. cerevisiae* K48L3 and K48L4 were isolated from YPH499. All of the *S. cerevisiae* strains were grown in complex YPD [yeast extract 2%, bactopeptone 4%, adenine solution (×100) 2%, uracil solution (×100) 2%, glucose 4%] at 30°C.

Cell Culture

A murine macrophage cell line, RAW 264.7, and murine fibroblast cell line, L929, were purchased from Korea Cell Line Bank (KCLB, Seoul, Korea). The cells were grown n an RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, Gibco BRL, Grand Island, U.S.A.), 100 U/ml of penicillin, and 100 μg/ml streptomycin.

UV Mutagenesis (Random Mutagenesis)

Yeast cells were grown as 5 ml cultures in YPD media until the mid logarithmic phase. The cultured yeast cells were washed twice in sterile water and irradiated on plates. Two-hundred μl of 10× diluted cell suspension was spread on each plate and exposed to 50 J/m^2 UV. The plates were incubated in the dark for at least 24 h to avoid photoreactivation [24]. The lethal rate was 99% in 50 J/m^2.

Screening of Cell Wall Mutants by Laminarinase Resistance

To isolate cell wall mutants, YPH499 was mutated randomly by UV irradiation as described above. Then, lyticase-resistant strains were selected by its resistance to laminarinase [endo-β(1,3)-glucanase] (Sigma Chemical Co., U.S.A.) [14, 21]. YPH499 was used as controls for the following experiments. Diluted cells were plated on YPD plates to determine the total initial count, T (cfu/ml). The cultured cells were washed twice with 0.1 M phosphate buffer (pH 7.0) and resuspended in Solution I (0.1 M phosphate buffer, 0.01 M EDTA, and 1% (v/v) 2-mercaptoethanol). The cells were incubated at 30°C for 30 min and then collected by centrifugation and resuspended in Solution II (1 M sorbitol, 0.01 M EDTA, 0.01 M phosphate buffer) containing 1 mg/ml laminarinase and incubated at 37°C for 30 min. The cells were harvested and washed three times with 1 M sorbitol. Osmotically sensitive protoplasts were lysed by resuspending the cells into distilled water and vortexed for 2 min. Cell suspensions were placed on YPD plates to determine the viable count, V (cfu/ml). The resistance is defined as the ratio of V/T.

Isolation of Cell Wall Mannoproteins

Cell wall mannoproteins from YPH499, K48L3, and K48L4 were isolated and extracted with SDS (sodium dodecyl sulfate) and laminarinase [15]. The cells in the late logarithmic phase were harvested and washed twice in 10 mM Tris-Cl (pH 7.4)–1 mM phenylmethylsulfonyl fluoride (buffer A). The harvested cells were resuspended in buffer A containing 2% SDS (1 g wet weight/10 ml) and were boiled for 10 min. Breakage of the cell was confirmed by microscopy, and the cell walls were washed three times in buffer A. The washed cell walls were finally digested in buffer A containing 1,200 units of glucanase (10 ml/g wet weight of cell wall) for 3 h at 30°C in an orbital incubator at 200 rpm. The extract was obtained by centrifugation of the above digest. Protein concentrations were determined with Bradford bioassay reagent with bovine serum albumin as a reference protein. Glucanase-extracted mannoproteins were further purified by ion-exchange and affinity chromatographies.

Mannoproteins were prepared through the binding characteristics of lectins (Fig. 1): Concanavalin-A (Con A) binds to α-D-glucosyl and α-D-mannosyl residues, but has no affinity for β-D-glucosyl groups [26]. Thus, total mannoproteins (glucanase-extracted mannoproteins) were loaded onto DEAE sephadex A-50, followed by concanavalin-A affinity column. Twenty ml of the Con A-Sepharose 4B gel was washed with 200 ml of washing buffer (20 mM Tris-HCl, pH 7.4; 0.5 M NaCl; 1 mM $MnCl_2$, $MgCl_2$, $CaCl_2$) to equilibrate the column and remove preservative. The mixture of mannoproteins and others (50 mg/5 ml of washing buffer) was applied and eluted with two to three bed volumes of washing buffer. The eluted mannoproteins were dialyzed against sterile water and lyophilized.

Separation of the Mannoprotein by Molecular Weights (Mw)

The molecular mass of β-glucan was measured and separated by high-performance liquid chromatography (HPLC) and fast-performance liquid chromatography (FPLC). Purified mannoproteins were analyzed and separated by GPC column (Ultrahydrogel 2000, Waters Corp., U.S.A.) at room temperature (25°C) at a flow rate of 0.3 ml/min with deionized and degassed water by HPLC.

FPLC was used to collect the mannoprotein fractions. The total mannoproteins were separated on DEAE anion-

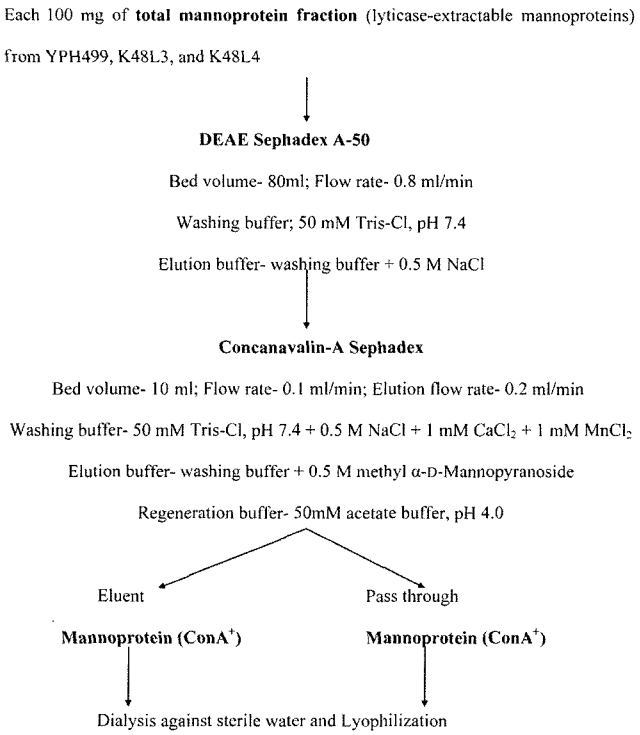


Fig. 1. Outline of chromatographic preparation of mannoproteins. Total mannoproteins (glucanase-extracted mannoproteins) were loaded onto a DEAE sephadex A-50 column following by concanavalin-A affinity column.

exchange chromatography (UNO-Q1 column, Bio-Rad Laboratories, U.S.A.) at room temperature (25°C) at a flow rate of 0.2 ml/min with 50 mM Tris-Cl (pH 7.4). The mannoproteins were fractionated with a linear gradient formed from 0 to 1 M NaCl. Five molecular weight markers [albumin bovine serum (66,000), alcohol dehydrogenase (150,000), β-amylase (200,000), apoferritin (443,000), thyroglobulin (669,000)] were used in this chromatography.

Quantification of Monosaccharides

HPLC was adopted to identify monosaccharides of total mannoprotein, concanavalin-A-eluted mannoprotein, and mannoprotein of concanavalin-A pass-through. Hydrolysis of the mannoproteins was carried out in 2 ml of 2 M trifluoroacetic acid (TFA) at 121°C for 90 min. Chromatography of samples was performed using HPLC. The monosaccharides liberated by acid hydrolysis (glucose, mannose) were separated on an Aminex HPX-87 K HPLC column equipped with a CarboPac PA guard column (Bio-Rad Laboratories, U.S.A.). Elution was performed at room temperature (25°C) at a flow rate of 0.5 ml/min with deionized and degassed water.

Bioassay of Nitric Oxide

The 24-h macrophage supernatants (100 μl) were assayed for nitric oxide by Griess reaction according to the microassay method described [3]. Briefly, an equal volume of Griess reagent (1% sulfanilamide/0.1% naphthylenediamine dihydrochloride/2.5% H_3PO_4) was incubated with macrophage supernatants treated by mannoproteins for 10 min at room temperature, and absorbance at 550 nm was measured in a micro ELISA reader. Nitric oxide concentration (nmol/well) was then calculated using $NaNO_2$ as a standard.

L929 Cytotoxicity Assay

TNF-α content in the medium was determined by a modified L929 cytotoxicity assay [12, 13, 17, 20]. Thus, murine L929 monolayers were grown to confluence in RPMI 1640 medium containing 10% FBS, harvested with trypsin, and plated on 96-well plates overnight at 37°C. The following day, the medium was replaced with RPMI 1640 medium containing actinomycin D (1 μg/ml). Recombinant murine TNF-α (Sigma) was used as a relative standard of bioactivity for these assays. Standards or samples were plated onto the L929 cells, and the cells were incubated for 18 h at 37°C. Thereafter, the medium was removed. The cells were stained with 0.5% crystal violet and determined at 490 nm. A standard concentration curve was prepared by using optical densities of the standards and used to calculate the TNF-α contents of the samples.

Assay of Phagocytosis

Phagocytotic activity was assayed by a method described previously [27]. Briefly, Raw 264.7 cells (1×10^5 cells/well) washed twice with 100 μl of RPMI 1640 were incubated for 30 min at 37°C with 1×10^7 of FITC-labeled *E. coli* (4×10^6 cells/well) (Molecular Probes, Eugene, U.S.A.) in RPMI 1640 containing 10% FBS in 96-well flat-bottomed tissue culture plates. The macrophages were then washed 3 times with warm PBS and solubilized by the addition of 100 μl of 50 mM sodium cholate. The fluorescence intensity was measured with a microplate reader with an excitation wavelength of 490 nm and an emission wavelength of 530 nm.

RESULTS AND DISCUSSION

Separation and Determination of the Mannoproteins from Yeast Cell Wall

To isolate mutants with a high yield of mannoprotein, wild-type strain YPH499 was mutated by UV irradiation, and the glucanase-resistant strains were then selected by screening the resistancy to laminarinase [endo-β(1,3)-glucanase]. The laminarinase activity and the lethal rate of the yeast were modulated by the amount of the enzyme used for the reaction. The lethal rate of the wild-type cells reached almost 100% with 0.2 units of laminarinase/10^5

Table 1. Laminarinase resistance of cell wall mutants.

Strains	Initial count (T) (cfu/0.1 ml)	Viable count (V) (cfu/0.1 ml)	Viable cells/ Total cells	Survival ratio (%)
YPH499 (W/T)	2.7×10^5	10	3.7×10^{-5}	0.0037
K48L3	2.9×10^5	2.62×10^3	0.94×10^{-2}	0.94
K48L4	2.5×10^5	1.58×10^3	0.63×10^{-2}	0.63

YPH499 was used as the haploid control strain. K48L3 and K48L4 were mutants derived from the wild-type YPH499 that overproduces cell wall mannoproteins. K48L3 and K48L4 exhibited particularly high resistancyies of 250-fold and 170-fold, respectively. The results represent the mean of two independent experiments±SD.

cells. *S. cerevisiae* K48L3 and K48L4 are the mutants isolated from YPH499 with high mannoprotein content. These mutant strains exhibited approximately 300-fold higher resistancy to laminarinase than the parental strain YPH499 (Table 1): K48L3 and K48L4 showed 250-fold and 170-fold higher resistancies, respectively. The yield of the mannoproteins in the mutants was 3-fold higher than that in the wild-type (Table 2). Mannoproteins of *Saccharomyces cerevisiae* were released by laminarinase, purified by concanavalin-A affinity and DEAE-triacryl anion-exchange chromatographies. In particular, the cell wall mutant K48L3 yielded 3-fold more total mannoprotein and 8-fold more mannoprotein ($conA^+$) than the wild-type.

When purified mannoproteins were analyzed and resolved by HPLC and FPLC gel filtrations (Fig. 2), seven proteins were found with molecular weights ($\times10^3$) of 695, 477, 404, 299, 274, 239, and 194 in YPH499 (wild-type) and 695, 473, 392, 277, 260, 230, and 192 ($\times10^3$) in K48L3. Mannoproteins of fractions 1, 2, 4, 5 (F1, F2, F4, F5) were eluted by concanavalin-A affinity chromatography following by DEAE-triacryl anion-exchange chromatography. Mannoproteins of F3, F6, F7 passed through the concanavalin-A affinity chromatography column. The K48L3 cell wall mutant had a higher amount of F1 with the highest Mw than the wild-type YPH499.

Carbohydrate analysis (Table 3) revealed that they contained mannose, glucose, and N-acetylglucosamine. Hydrolysis of the mannoproteins was carried out in 2 ml of 2 M trifluoroacetic acid (TFA). Total (glucanase-extractable)-, mannoprotein ($conA^+$)-, and mannoprotein ($conA^-$)-mannoprotein were loaded on an Aminex HPX-87K HPLC column equipped with a CarboPac PA guard column (Bio-Rad Laboratories, U.S.A.). Elution was performed at room temperature (25°C) at a flow rate of 0.5 ml/min with deionized and degassed water. The glycosylation part of total mannoproteins and con-A-eluted mannoproteins were hydrolyzed to glucose (20 min), N-acetylglucosamine (23 min), and mannose (25 min). The ratio of mannose among monosaccharides was about 67:80 (total mannoprotein: concanavalin-A-eluted mannoprotein), respectively. The mannoprotein ($conA^+$) consisted mostly of mannose (80±1%) and a small percentage of glucose (14±1%), depending on the yeast strain.

Analysis of Immune Activity of Mannoproteins

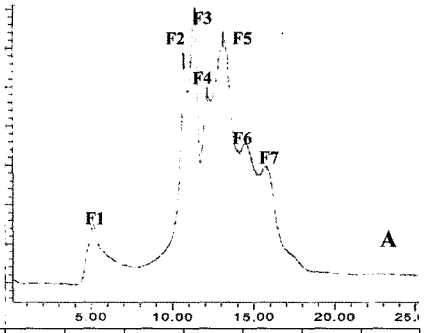
Nitric oxide is an important biologic mediator in several cell types, including macrophages [16, 25, 34]. Macrophages released significant amounts of nitric oxide when incubated with mannoproteins. For the experiments described above, nitric oxide levels in the supernatant were determined 24 h after the addition of macrophage activator, mannoproteins. As shown in Fig. 3, macrophages were induced to release nitric oxide when incubated with each mannoprotein fraction. All of the total mannoproteins of YPH499, K48L3, and K48L4 (10–400 μg/ml) induced nitric oxide secretion from RAW 264.7 cells in a dose-dependent manner (data not shown). K48L3, the cell wall mutant, at concentration of 400 μg/ml was approximately 2 times more effective in inducing nitric oxide than wild-type (YPH499) and positive control (lipopolysaccharide, LPS). F1 and F2 among the mannoprotein fractions revealed higher nitric oxide secretion activity than the others (Fig. 3). In particular, F1 showed the highest immune-enhancing activity, and K48L3 had more F1 than wild-type.

Table 2. Yield of yeast cell wall mannoproteins.

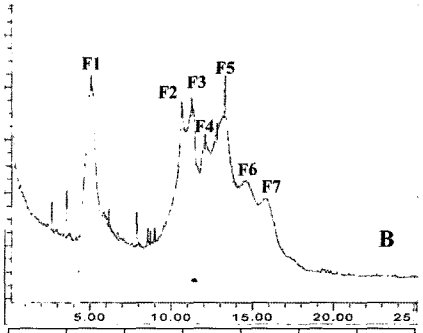
Strains	Weight of total mannoproteins (μg/100 mg dry weight of yeast)	Weight of mannoprotein ($conA^+$) (μg/100 mg dry weight of yeast)	Weight of mannoprotein ($conA^-$) (μg/100 mg dry weight of yeast)
YPH499	725	256	348
K48L3	2,255	1,793	446
K48L4	825	285	462

Protein concentrations were determined with Bradford bioassay reagent with bovine serum albumin as a reference protein. The cell wall mutant, K48L3, yielded 3-fold more total mannoprotein and 8-fold more mannoprotein ($conA^+$) than those of the wild-type. The results represent the mean of three independent experiments±SD.

A. Total mannoproteins

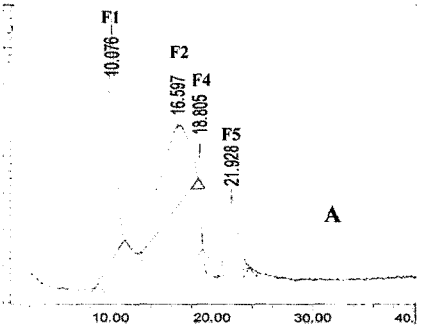


F.No.	F1	F2	F3	F4	F5	F6	F7
Mw ($\times 10^3$)	695	477	404	290	274	239	194

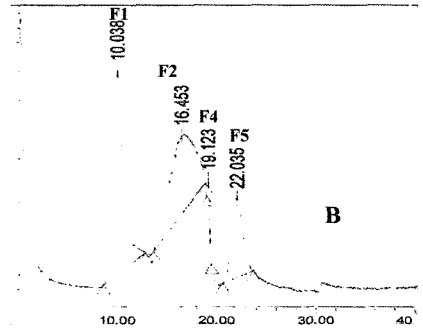


F1	F2	F3	F4	F5	F6	F7
696	474	392	277	260	230	192

B. Con-A-eluted mannoproteins

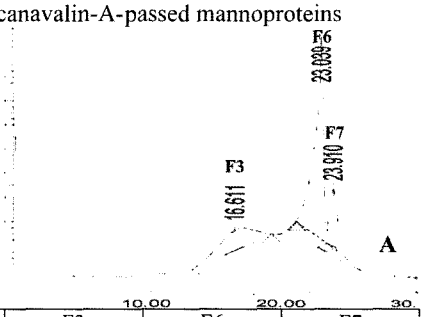


F.No.	F1	F2	F4	F5
Mw ($\times 10^3$)	695	477	290	274

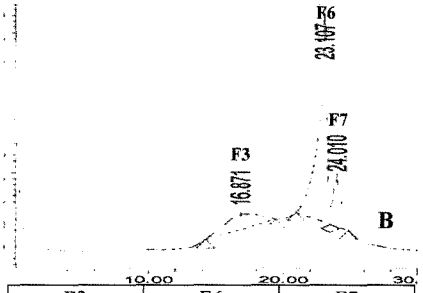


F1	F2	F4	F5
696	474	277	260

C. Concanavalin-A-passed mannoproteins



F.No.	F3	F6	F7
Mw ($\times 10^3$)	404	239	194



F3	F6	F7
392	230	192

Fig. 2. GPC chromatograms of mannoproteins.
Total (glucanase-extractable) mannoprotein, mannoprotein (conA$^+$), and mannoprotein (conA$^-$) were loaded on GPC columns (Ultrahydrogel 2000, Waters Corp., U.S.A.) at room temperature (25°C) at a flow rate of 0.3 ml/min with deionized and degassed water by HPLC. A, Chromatograms of YPH 499 (wild-type); B, Chromatograms of K48L3 (cell wall mutant).

Maximal TNF-β release occurred at higher concentrations of mannoprotein (10 to 400 μg/ml) (data not shown). There was no suppression of the TNF-α activity by mannoproteins. With regards the biologic implications of the observations described above, the present study demonstrated that mannoproteins stimulated the release of TNF-α, regardless of high concentration. F1, F2, and F3 showed higher TNF-α secretion activity than the others (Fig. 4). In particular, F1 showed the highest immune-enhancing activity. In addition, K48L3 had more F1 than wild-type. The amount of TNF-α secreted by F1 of both YPH499 and K48L3 was 1,300 pg and 980 pg, respectively.

Table 3. Monosaccharides of total mannoproteins and mannoprotein (conA$^+$).

Strain	Class of mannoprotein	Rate of glucose (%)	Rate of AcNGlc (%)	Rate of mannose (%)
YPH499	Total mannoproteins	18.37	13.90	67.72
	mannoprotein (conA$^+$)	14.57	6.21	79.22
K48L3	Total mannoproteins	18.87	13.40	67.73
	mannoprotein (conA$^+$)	13.91	5.30	80.79
K48L4	Total mannoproteins	21.86	14.83	63.30
	mannoprotein (conA$^+$)	14.49	6.11	79.40

Total (glucanase-extractable) mannoprotein and mannoprotein (conA$^+$) were loaded onto an Aminex HPX-87K HPLC column equipped with a CarboPac PA guard column (Bio-Rad Laboratories, U.S.A.). The glycosylation part of total mannoprotein and mannoprotein (conA$^+$) was hydrolyzed to glucose (20 min), N-acetylglucosamine (23 min), and mannose (25 min).

Phagocytosis has long been recognized as a primary function of macrophages, and its role in the process of macrophage differentiation has been studied [28–30, 35]. In the present study, macrophages were tested for their ability to phagocytize FITC-labeled *E. coli* bacteria, and the result is displayed in Fig. 5. At a 10:1 bacteria/RAW 264.7 (murine macrophage) ratio, the mannoproteins-treated cells exhibited greater antibacterial activity than the control cells, in terms of both the percentage of cells engaged in phagocytosis and the mean number of bacteria

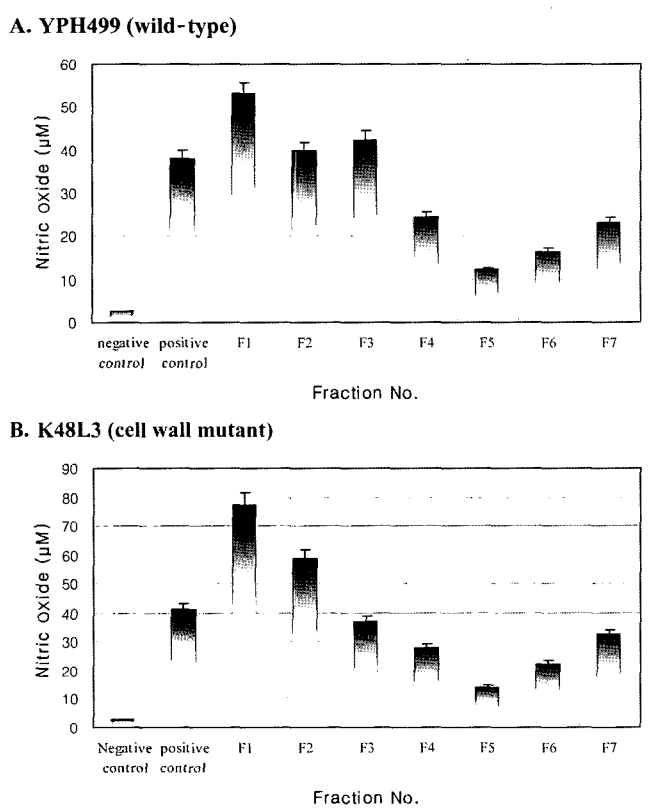


Fig. 3. Nitric oxide bioassay of mannoprotein fractions.
RAW 264.7 (Murine Mφ, 1×10^6 cells/each well) pretreated with mannoprotein fractions of YPH499 and K48L3 were incubated for 21 h. Supernatants were mixed with the same volume of Griess reagent. NO product was measured with a microplate spectrophotometer at 520 nm. LPS (1 μg/ml) was used as a positive control and nitric oxide values were 41.21 (±5.5) μM [A] and 38.53 (±3.7) μM [B]. The results represent the mean of four independent experiments±SD. *Significant differences between wild-type (YPH499) and mutant (K48L3) at the $P<0.05$ level.

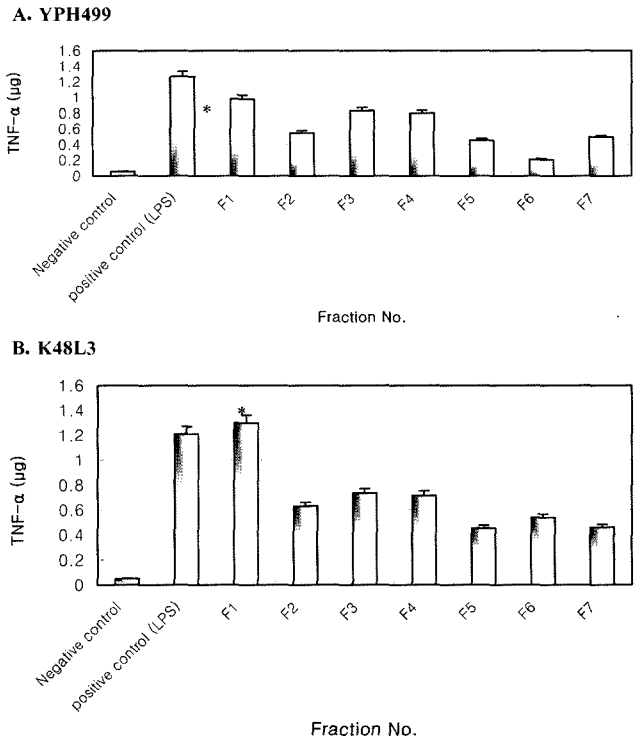


Fig. 4. Effect of the mannoprotein fractions on TNF-α secretion of murine macrophage cell line, Raw 264.7.
Mannoprotein fractions were, respectively, added to Raw 264.7 (4×10^5 cells/ml) cells and incubated for 21 h. The supernatants were harvested and the amount of TNF-α was measured using L929 bioassay. The TNF-α concentrations in the media of macrophages incubated in the absence of mannoproteins were 0.05 (±0.01) ng [A] and 0.07 (±0.02) ng [B]. LPS (1 μg/ml) stimulation of macrophages was used as a positive control and the TNF-α values were 1.29 (±0.18) ng [A] and 1.21 (±0.12) ng [B]. The results represent the mean of four independent experiments±SD. *Significant differences between wild-type (YPH499) and mutant (K48L3) at the $P<0.05$ level.

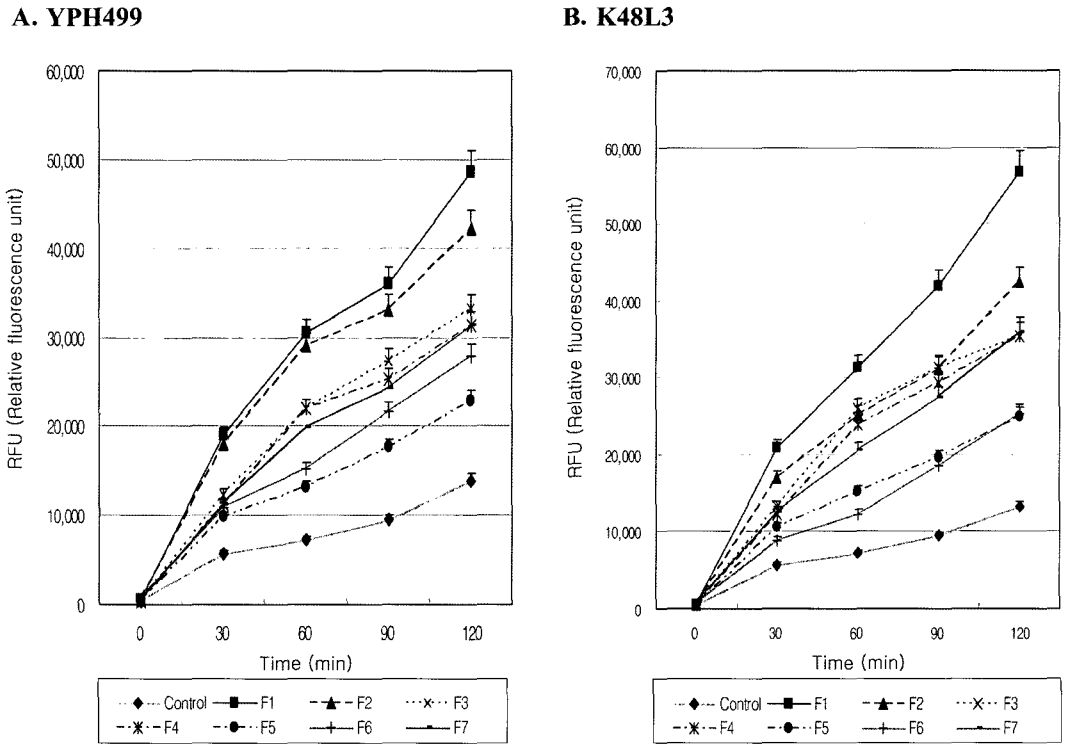


Fig. 5. Effect of the mannoprotein fractions on phagocytosis by murine macrophage cell line, RAW 264.7.
Murine macrophage cell line, RAW 264.7, was stimulated with seven mannoprotein fractions, respectively. FITC-labeled *E. coli* was added to RAW 264.7 at the ratio of 1:20 (macrophage : bacteria). All experiments were performed in triplicate. *Significant differences between wild-type (YPH499) and mutant (K48L3) at the $P<0.05$ level.

phagocytized per cell. These results were reproducible in several independent experiments. All the mannoprotein fractions had a higher macrophage antibacterial activity than the negative control: Specifically, F1 and F2 among the cell wall mannoproteins of K48L3 enhanced and upregulated macrophage phagocytic activity approximately four-fold.

It was demonstrated in the present study that the cell wall mutants yielded 3-times more cell wall mannoprotein. The general composition of the polysaccharides of mannoproteins of mutants K48L3 and K48L4 was similar to that of the wild-type, consisting of mannan, glucose, and glucosamine. The chemical structures of the mannoproteins from *S. cerevisiae* have been well characterized [6, 7]. The mannoprotein (conA$^+$) from cell wall mutant K48L3 comprised approximately 1.8% of the cell dry weight. This value was about 7 times higher than the value of the wild-type. It is also important to note the possibility that these higher values of cell wall occurred by UV random mutation. This consideration is important if the goal is to maximize cell wall yields, as would be the case in commercial situations.

Macrophages are known to play a central role in the generation of specific and nonspecific immunity. Upon interaction with yeast cell wall components, macrophages bind and phagocytize yeast and are stimulated to release inflammatory mediators, including tumor necrosis factor alpha (TNF-α) and nitric oxide [8, 12]. We evaluated the nitric oxide and TNF-α production, and phagocytosis in order to demonstrate whether the mannoproteins of cell wall mutants really exhibit an immune-enhancing characteristic, and also to find out which mannoprotein separated from cell wall mannoprotein by molecular weight had higher immunoactivity.

Yeast cell wall mannoprotein has a long history as nonspecific biological modulators. Recognition of mannoprotein is an important mechanism of host interaction with various pathogens [12]. In this study, these mannoproteins were isolated and fractionated from *Saccharomyces cerevisiae*. In particular, fraction F1 among seven mannoprotein fractions showed significant stimulation of phagocytic activity as well as potentiation of synthesis and release of nitric oxide and TNF-α. The yeast cell wall mannoproteins showed a broad spectrum of immune activity. Moreover, the mannoproteins used in this study were highly water soluble. It may provide a therapeutic approach to preventing and treating oral bacterial infections by upregulating host antibacterial defense mechanisms. However, more study is required to establish the full biological activities and structure of the mannoproteins and to evaluate their industrial or medicinal applications.

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