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Review article

Yeast cell wall mannan structural features, biological activities, and production strategies

Kwang-Rim Baek [a,1], Sudha Rani Ramakrishnan [b,c,1], Soo-Jung Kim [b,**], Seung-Oh Seo [a,d,*]

[a] Department of Food Science and Biotechnology, Seoul National University of Science and Technology, Seoul, 01811, Republic of Korea
[b] Department of Integrative Food, Bioscience, and Biotechnology, and Research Center for Biological Cybernetics, Chonnam National University, Gwangju, 61186, Republic of Korea
[c] Department of Biotechnology, Anna University, Chennai, 600025, India
[d] Research Institute of Food and Biotechnology, Seoul, 01811, Republic of Korea

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ABSTRACT

Mannan and outer structural yeast cell wall polysaccharides have recently garnered attention for their health defense and cosmetic applications. In addition, many studies have confirmed that yeast cell wall mannans exhibit various biological activities, such as antioxidant, immune regulation, reducing hyperlipidemia, and gut health promotion. This paper elucidates yeast cell wall mannan structural features, biological activities, underlying molecular mechanisms, and biosynthesis. Moreover, mannan-overproducing strategies through yeast strain engineering are emphasized and discussed. This review will provide a scientific basis for yeast cell wall mannan research and industrial applications.

1. Introduction

Yeast is a unicellular fungus utilized extensively in feed, food, and beverage industries [1,2], generating considerable scientific intrigue as it is inexpensive and easy to culture [3]. In addition, yeast can ferment at high cell densities, which is advantageous for producing target compounds with limited time and resources [4]. Yeast cell wall constituents are bioactive molecular sources that provide functional properties to fermented products [5]. The yeast cell wall constitutes 15–30% of cellular dry weight and 25–50% volume, supplementing β-1,3-glucan (240 kDa), β-1,6-glucan (24 kDa), mannan bound with protein (100–200 kDa), and chitin (25 kDa) [6] (Fig. 1). The composition of yeast cell wall is predicated by strain type, physiological state, cell growth stage, and cultivation conditions [6]. Cell wall mutants also expressed altered relative layer thicknesses and organization [7].

Yeast cell wall glucan and mannan have been widely studied and gained commercial interest for their extensive industrial applications [8]. Although food and feed industries initially use yeast polysaccharides as dietary fibers, emulsifying agents, or fat replacers, there is an increasing interest in potential pharmaceutical and biomedical applications, with an emerging focus on

* Corresponding author. Department of Food Science and Biotechnology, Seoul National University of Science and Technology, 232 Gongneung-ro, Nowon-gu, Seoul, 01811, Republic of Korea.
** Corresponding author. Department of Integrative Food, Bioscience, and Biotechnology, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju, 61186, Republic of Korea.
E-mail addresses: bioksj@jnu.ac.kr (S.-J. Kim), drsos@seoultech.ac.kr (S.-O. Seo).
[1] Kwang-Rim Baek and Sudha Rani Ramakrishnan contributed equally to this work.

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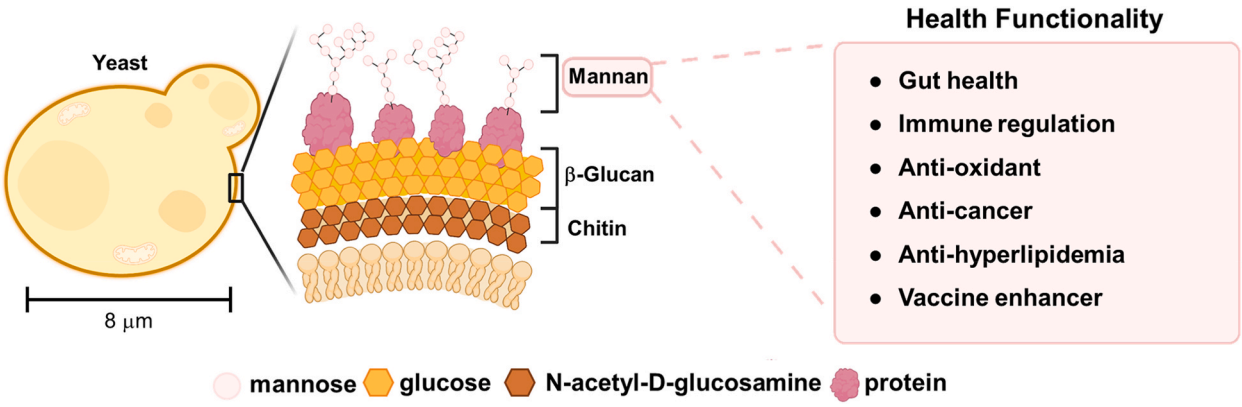

Fig. 1. Yeast cell wall structures and biological activities of mannan fraction. This figure was created with BioRender (https://biorender.com/).

immunology, tissue engineering, vaccines, and drug delivery [9]. According to the latest Facts and Factors (Report 2021) market research publication, a demand analysis revealed that the global specialty yeast market size and share revenue were valued at approximately 2.9 billion USD in 2020 [10]. Furthermore, it is projected to reach 4.3 billion USD by 2026, with a 7.9% compound annual growth rate from 2021 to 2026 [10]. The increased demand for natural food ingredients is expected to amplify the specialty yeast industry's market growth. The yeast market can be further divided by type: yeast extract, yeast β-glucan, yeast autolysates, and hydrolyzed yeast; species: *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Kluyveromyces*; and application segment: feed, food, savory snacks, cheese, bakery, confectionary, ready-to-eat, functional foods, biofuels, bioethanol, and beverages [10].

Among yeast cell wall constituents, mannan or mannan-oligosaccharide (MOS) is regularly used in the feed industry [11]. For instance, livestock that consume MOS from an early-stage exhibit remarkably improved immune system [12]. In addition, mannan extracts have also been studied for their beneficial effects on humans. For example, one study noted that mannan aid probiotic bacteria survival in yogurt [13], while a clinical study reported yeast mannan supplements improved gut microbiota and skin dryness in healthy subjects [14]. Furthermore, additional studies have focused on applying yeast mannan to biosensors [15], vaccine enhancers, and delivery systems [16,17].

Recent research studies are immersed in selecting high mannan-producing strains and applying metabolic engineering approaches to enhance their accumulation in the cell wall [18–20]. Similarly, this review focuses on mannan derived from yeast cell walls and its structural properties, biological activities, and production methods.

2. Mannan and other yeast cell wall polysaccharide structures and usage

2.1. Cell wall polysaccharide structures

Electron microscopic cell wall analysis revealed a layered structure with an inner layer approximately 200 nm thick, comprising glucan and chitin, accounting for about 50–60% of the cell wall dry weight [6]. Mannan emanates from the outer cell surface layer and is responsible for approximately 30–50% of cell wall mass [21]. Mannan binds to the multiple phosphodiester bridges on carbohydrate side chains of surface proteins [21] and mannoproteins are either indirectly or directly covalently linked to the β-1,3-glucan-chitin network [6] (Fig. 1).

β-glucan chains in the cell wall are predominantly linked in flexible β-1,3 structures to extend in moderately branched configurations [6,21]. In addition, β-1,6-linked glucose residues compose approximately 3–4% of β-1-3-glucan in stationary phase cells [6]. The mature β-(1–3, 1–6)-glucan is a highly branched, water-soluble polymer with approximately 150 glucose monomers [22]. β-glucan is recognized as immunomodulatory, enhancing the body's immune response. Currently, studies are attempting to increase the biological activity of β-glucan from yeast cell wall [24].

Chitin subsists as linear chains around the septal region, and bud scars within the mother cell's lateral walls [25]. Notably, chitin isolated from bud scars comprises about 190 N-acetylglucosamine monomers [26] Chitin contributes significantly to the mechanical strength and flexibility of the cell wall, playing a crucial role in maintaining cell shape [27]. The complex interactions between chitin and other cell wall components are essential for the overall structure and function of the cell wall, influencing the integrity of the cell wall and its responsiveness to environmental changes [27].

In addition, the mannoprotein that forms the outer cell wall layer is highly glycosylated with a carbohydrate fraction over 90% (w/w) and can control cell wall porosity [23,28]. This ability is because mannoproteins have highly branched carbohydrate side chains linked to asparagine residues, forming rigid rod-like polypeptide backbone regions when exposed to serine and threonine residues [6]. An important structural feature of cell wall mannoprotein is the presence of multiple phosphodiester bridges on carbohydrate side chains [21]. These phosphorylated mannose residues maintain cell wall integrity, contributing to the stable structural and functional aspects of the cell surface. However, exposure to acidic conditions can lead to the hydrolysis of phosphodiester bonds, rendering them unstable [29]. Mannan is the outer mannoprotein layer, carrying highly mannosylated O- and asparagine-N- linked glycan with

glycosylphosphatidylinositol [30]. Mannan are polysaccharides, or mannose polymers ($C_6H_{12}O_6$), the generic name for polysaccharide moiety of glycoprotein. In plants, mannan exhibits considerable structural variation, serving as a key member of the hemicellulose family. plants' mannan can be categorized into four subfamilies: linear mannan, glucomannan, galactomannan, and galactoglucomannan [31]. Plants' mannan is present in different forms but consistently exhibits a mannose residue-only β-1,4-linked backbone, a glucose and mannose residue combination, and α-1,6-linked galactose residue side chains [31]. Yeast mannan has an α-1,6-linked backbone and α-1,2- and α-1,3-linked mannose branches [23], which differs from plants' mannan [32] (Fig. 2). More complex structure of cell wall mannan can be formed by various glycosidic and phosphodiester bonds between monosaccharide units [33,34].

2.2. Cell wall polysaccharide usage

Yeast cell wall components, including mannan, β-glucan, and chitin, have been evaluated for commercial applications (Table 1). Termed "Generally Recognized as Safe (GRAS)" by the Food and Drug Administration (FDA), β-glucan is used as a texturing agent in the food industry [35,36], bio-based film development for food contact packaging materials, and medical and material science applications [37]. In addition, depending on the β-glucan nature, β-glucan intake decreases plasma cholesterol and immune system stimulation [35,38].

Mannan is composed of mannose units, and their structure can vary significantly in terms of molecular weight, degree of branching, and the presence of side chains [39]. For example, the degree of branching in mannan molecules can affect their ability to interact with immune cells [39]. More highly branched mannans might exhibit enhanced interactions with specific receptors on immune cells, potentially leading to stronger or more targeted immune responses [39]. Similarly, the molecular weight of mannan can influence its functionality as a prebiotic [40]. Mannan is a bioactive polysaccharide prominent in various sectors due to its exploitable biodegradable properties [41]. Furthermore, its amphiphilic nature serves as a bio-emulsifier [42] and is also used as a bioactive material in health-related applications. For example, mannans conjugated to vaccine preparations are already in clinical practice [43]. In a recent European oral drug delivery review, mannan-based nanogels were considered a novel approach for oral labile molecule delivery [44]. Mannan extracted from yeast has garnered increasing focus because the yeast extract sector produces extensive cell wall by-products, establishing a cheap source for obtaining mannan [45]. In addition, long mannan chains are hydrolyzed into short chains known as MOS. Due to their bioactivity, yeast-derived MOS are used as prebiotics in animal husbandry and nutritional supplements [46].

3. Biological activities of mannan

3.1. Gut health

The gut is responsible for nutrient digestion and absorption, protection against pathogens or toxins, and hosts a large microbiome population [47]. Moreover, the gut can regulate the body's immune system, and inflammation [48], and influence the gut-brain axis [49]. Thus, healthy gut is considered an important factor in maintaining a status of well-being [50]. Healthy gut can be evaluated by

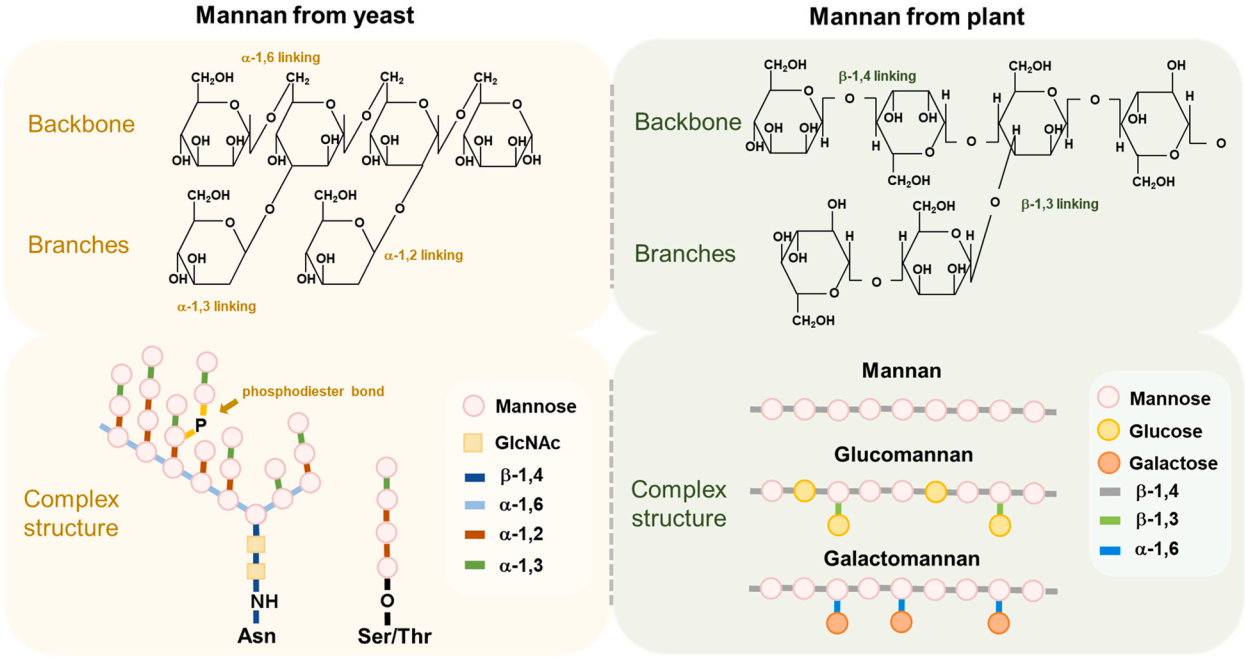

Fig. 2. Yeast mannan and other cell wall polysaccharide structures. This figure was created with BioRender (https://biorender.com/).

Table 1
Applications of yeast cell wall-derived components (mannan, β-glucan, and chitin).

Yeast cell wall components	Category	Applications	References
Mannan	Antibiotics	Avert intestinal pathogen adherence, such as *E. coli* and certain *Salmonella* species, to the gut mucosa, subsequently aiding many gut-related disease treatments.	[84]
	Feed additives	Effectively changes the gut's bacterial ecology.	[144]
		Hydrolyzes antinutritional elements and produces beneficial MOS.	[93]
		MOS acts as a prebiotic and dietary fiber that lessens intestinal disorder incidence.	[145]
		Improves hens' egg-laying performance.	[146]
		Antibiotic-free diet for dairy cows; increases rumen pH during subacute ruminal acidosis; inflammation reduction; high colostrum production and fat content in milk.	[147]
		Nile Tilapia (*Oreochromis niloticus*, juvenile): MOS (1, 8, and 15 g/kg of diet) modulates intestinal microbiota and stimulates immunity by elevating white blood cells and lysozymes.	[148]
		Nile Tilapia (*O. niloticus*, larvae): MOS at 0.34% of diet improved feed conversion and increased intestine length, villus height, and intestinal villus density.	[149]
		Tilapia hybrid (*O. niloticus* x *Oreochromis aureus*, juvenile): MOS (1.5, 3, 4.5 g/kg of diet) increased body protein and MOS concentration; average villus length was greater in fish fed 1.5 g of MOS/kg diet.	[150]
	Food industry	Gel formation, edible films, stiffeners, viscosity modifiers, stabilizers, texture improvers, water absorbents, and prebiotics in dairy, bakery, seasonings, diet foods, and coffee whiteners, etc. Nutrient fortification, diet enhancement, thickening agent degradation, biomass conversion to bio-sugar for fermentation, and fruit and vegetable maceration.	[151]
		Mannanases improve fruit juice clarity, viscosity reduction, instant coffee extract clarification, vegetable oil extraction from copra meal, and slime control agents.	[152]
		Enology industry: ochratoxin A adsorption, complexation with phenolic compounds, increases malolactic bacteria growth, tartrate salt crystallization inhibition, haze formation prevention, and aromatic component reinforcement.	[153]
	Healthcare	Nutraceuticals that control obesity and body weight, constipation alleviation, prevents diarrhoea, checks gut-related diseases inflammation, diverticular disease management, balances the intestinal microbiota, immune system modulator, and reduces colorectal cancer risk.	[151]
		Candida utilis glucomannan in rheumatoid arthritis treatment.	[154]
		Aloe vera gel's anti-fungal activity, hypoglycaemic effects, wound healing, anti-inflammatory, anti-cancer, and immunomodulation.	[155]
β-glucan	Feed additive	Nile Tilapia (*O. niloticus*, juvenile): prebiotic β-polo (β-glucans 1.5 mL/kg of diet) improved performance, immunity, and *Pseudomonas fluorescens* resistance.	[156]
		Nile Tilapia (*O. niloticus*, juvenile): both β-glucans (Biorigin: 0.1 g of BG1/kg of diet and 0.1 g BG2/kg of diet) promoted resistance to *Streptococcus agalactiae*. BG2 improved performance.	[157]
		Pacu (*Piaractus mesopotamicus*, juvenile): β-glucans (BG1) and β-glucan-based products: 1.3 and 1.6 β-glucans (BG2) prevented *Aeromonas hydrophila* hypoxia and infection (1×10^6 CFU). Concentrations: 0.5% BG1 for 10 days and 0.1% for 15 improved leukocyte respiratory activity.	[38]
	Food industry	Source: *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*; anomeric glucose monomers arrangement for linear and branching α-,β-glucan differentiation, and α-β-mixed glucans with varying glycoside linkages, locations, and molecular weights; anticancer, immune-modulating, and anti-inflammatory; gelation, viscosity, and solubility in water.	[158]
		Flavour enhancement and *S. cerevisiae*-derived β-glucans in drinks (1.3 g/kg), powdered milk (25.5 g/kg), biscuits (6.7 g/kg), breakfast cereals (15.3 g/kg), and dairy (up to 3.8 g/kg).	[159]
		Thickener, water-holding agent, oil-binding agent, or emulsifying stabilizer in food products such as soups, sauces, desserts, and salad dressings.	[160]
		Texturing agents, bio-based films for food contact packaging materials, and polysaccharide nanocrystals.	[37]
		β-glucan (65–125 mg) in bread darkens the crumb, enlarges the crumb, crust springiness, and impacted the volatile profile with significant hexanal increment.	[161]
		β-glucan from Brewers' spent yeast is used in bread to brown the crust.	[162]
		β-glucan (0.75% w/w of wheat flour) from Brewers' spent yeast in bread darkened the crumb, enlarged augmented crumb, crust springiness, and incremented total dietary fibers.	[163]
		β-glucan (2%) in cookies improved the sensorial attributes and antioxidants.	[164]
		β-glucan (0.3%, w/w) from Brewers' spent yeast in yogurt preserved sensory quality and structure stability.	[165]
		β-glucan (0.2–0.8% w/w) from Brewers' spent yeast in skimmed-milk yogurt reduced fermentation time and improved textural properties.	[166]
		β-glucans (0.5–1% w/w) from *S. cerevisiae* in skimmed-milk yogurt improved firmness and a more stable microstructure.	[167]
		β-glucan (1.5–3% w/w) in meat products increased emulsifying capacity, improved water holding capacity, and emulsion stability.	[168]
	Microparticles	*S. cerevisiae*-derived oral delivery platform.	[169]
	Medicine	Decreased plasma cholesterol and immune system stimulation.	[37]
		Novel vaccine design.	[75]

(*continued on next page*)

Table 1 (*continued*)

Yeast cell wall components	Category	Applications	References
Chitin	Biomedical, agriculture, and food	*S. cerevisiae*-derived; N-acetyl-D-glucosamine β-(1, 4) linked polymer; biodegradable, biocompatible, nontoxic, and antibacterial characteristics; antibacterial and cell culture roles.	[170]
Mixture	Feed additive	Nile Tilapia (*O. niloticus*, juvenile): MOS and β-glucans (0.15% Power top/kg) altered blood parameters, improved performance, and *A. hydrophila* resistance.	[171]
		Nile Tilapia (*O. niloticus*, juvenile): MOS (180 g/kg of diet) and β-glucans (1.5 and 3 g/kg of diet) improved performance, survival, and immune system (resistance to *Yersinia ruckeri*); increased protein and lipids, villi height, goblet cell number, and intraepithelial lymphocytes.	[172]

Table 2
Biological activities of yeast mannan.

Biological activity	Strain	Mannan type	Experimental model	Experimental results	Reference
Gut health	*Saccharomyces cerevisiae*	Mannan	Healthy human fecal samples	Increased beneficial bacteria abundance and improved pH condition in the gut	[53]
	Saccharomyces cerevisiae	MOS	Broilers	Increased *Bifidobacteria* and *Lactobacilli* abundance and ceca villi elongation	[63]
	Saccharomyces cerevisiae	Mannan	Broilers	Decreased inflammatory factors and improved the tight junction protein, occludin	[57]
	Saccharomyces cerevisiae	MOS	Piglets	Increased length of the jejunal villi and decreased inflammatory factors, TNF-α	[58]
	Saccharomyces cerevisiae	Mannan	Yong broiler chicken	Genome transcriptional changes in intestine	[84]
	Saccharomyces cerevisiae	Mannan	The human colon cancer, HT-29 cell	Decreased *E. coli* adhesion to HT-29 and inflammatory cytokines TNFα and IL-1β production	[64]
	Saccharomyces cerevisiae	Mannan	Healthy female human fecal samples	Improved beneficial bacteria abundance in feces	[14]
	Saccharomyces cerevisiae	MOS	Buffalo	Increased *lactobacilli* and *Bifidobacterial;* decreased *E. coli*	[61]
	Saccharomyces cerevisiae	Mannan	Gnotobiotic mice	Increased *B. thetaiomicron*	[51]
	Candida albicans	Mannan	Epithelial rabbit cells	Displaced piliated *E. coli*	[62]
	Saccharomyces cerevisiae	Mannan	*In vitro* assay	Identify binding of cell wall mannan to pathogens, *E. coli* and *S. typhimurium*	[54]
	Saccharomyces cerevisiae	Mannan rich fraction	*In vitro* assay	Reduced antibiotic resistant-*E. coli* proliferation	[65]
	Saccharomyces cerevisiae	MOS	Rabbit	Suppressed T-2 toxin adsorption	[67]
Immune-boosting	*Saccharomyces cerevisiae*	Mannan	Primary ovine ruminal epithelial cell	Induced SBD-1 expression and activated MAPK and NF-κB pathways	[71]
	Saccharomyces cerevisiae	MOS	Chicken	Induced IgA secretion	[72]
	Candida albicans	Manno-protein	Murine macrophage cell, Ana-1	Promoted NO and ROS phagocytosis and production	[74]
Antioxidant	*Saccharomyces cerevisiae*	Mannan	*In vitro* assay	Removed free radicals	[82]
	Candid utilis	Glucomannan	*In vitro* assay	Measured superoxide anion radical scavenging activity	[83]
	Kluyveromyces marxianus	Mannan	*In vitro* assay	Chelated high copper and iron levels	[90]
	Commercial Yeast	Mannan	*In vitro* assay	Inhibited production and removed free radicals	[93]
	Saccharomyces cerevisiae	MOS	Broiler jejunum	Induced antioxidant-related genes	[84]
Other biological activity	*Kluyveromyces marxianus*	Mannan	High-cholesterol mice	Decreased the total cholesterol levels in the plasma and liver	[86]
	Candida albicans	Mannans	Acute hyperlipidemia mice	Lowered blood LDL cholesterol and triglycerides	[87]
	Saccharomyces cerevisiae	Mannan	Mice with tumor	Elevated peritoneal cell proliferation and lysosomal activity	[89]
	Kluyveromyces marxianus	Mannan	Tumor cell, HEP-G2	Inhibited proliferation of tumor cell	[90]

Ana-1: Antinuclear antibody – 1; C57BL/6: C57 black 6; SBD-1: Substrate binding domain – 1; MAPK: Mitogen-activated protein kinase; NF-κB: Nuclear factor kappa B; NO: Nitric oxide; ROS: Reactive oxygen species; IL: Interleukin; TNF-α: Tumor necrosis factor; MOS: Manno-oligosaccharides.

two indicators: the integrity of the gut barrier and a balanced gut microbiome [50]. Several studies have reported that yeast cell wall mannan promotes gut and microbiota health in humans and animals, such as chickens, calves, horses, pigs, and fish [51,52] (Table 2). Mannan from yeast cell wall can function as a protectant of gut barrier, prebiotic and anti-bacterial agent [53,54].

Damage to the integrity of the gut barrier results in the weakening of the tight junction between enterocytes [55]. The damage in the enterocyte adversely affects defense function [55]. In the damaged gut barrier, macromolecules such as toxins and bacteria in the gut can enter the blood directly and cause various inflammatory reactions [56]. Mannan from the yeast cell wall may act as a protective agent for the gut barrier [57,58]. Mannan of the yeast cell wall improved inflammation-related gene expression in the ileum and tight junction proteins in broiler ileum [57]. Broilers fed a diet with or without MOS were orally infected with *E. coli* [57]. The *E. coli* infection increased the expression of inflammatory factors such as NF-κB and IL-1β, decreased the expression of anti-inflammatory factor such as IL-10 and tight junction protein such as occludin [57]. Broilers fed MOS had restored expression of inflammatory factors and increased expression of anti-inflammatory factors [57]. Also, the expression of occludin was restored by MOS treatment [57]. In another study, Agazzi et al. (2020) demonstrated the effects of MOS on gut health by evaluating the villus length and inflammatory response in piglets [58]. Piglets fed diets with or without MOS were raised for 36 days. As a result, the length of the jejunal villi of MOS-fed piglets was significantly increased, and the expression of the TNF-α gene, an inflammatory factor in the gut mucosa, was decreased [58]. The results of these studies suggest that mannan of yeast cell wall can promote morphological and immunological improvement in the gut.

Maintaining a balanced gut microbiome also can affect the intestinal defense system through numerous mechanisms [50,59]. Gut microbiome continuously interacts with enterocyte through enterocyte pattern recognition receptors (PRR) [60]. Gut microbiome can induce expression of defensins and the immune system to target potential invaders through interaction with enterocyte in the gut [59].

Numerous studies have investigated prebiotic effects of mannan in animals and human models (Table 2). In particular, Oba et al. (2020) collected fecal samples from healthy subjects and used *in vitro* fermentation systems to examine these prebiotic effects [53]. Mannan aided beneficial bacteria proliferation, such as *Bacteroides,* and improved gut pH conditions, indicating mannan's potential as a novel prebiotic [53]. In human models, yeast cell wall mannan increased the *Bacteroides thetaiotaomicron* population, a recognized probiotic in the gut [51]. Notably, as *B. thetaiotaomicron* secretes enzymes to digest and metabolize α-mannan in the human gut, *B. thetaiotaomicron* may be the dominant gut microbiota species when mannan is present [51]. This result highlights the adaptation of human gut microbes to utilize yeast mannan as a food source and identifies a specific mechanism through which mannan is metabolized. In one clinical study, 110 healthy female subjects 30–49 years old were supplemented with yeast mannan for eight weeks [14]. Microbiota analyses revealed that yeast mannan intake selectively increased relative *B. thetaiotaomicron* and *Bacteroides ovatus* abundance and ameliorated subjective skin dryness without any side effects [14]. These results propose that yeast cell wall mannan intake supports beneficial *Bacteroides* and improves intestinal and skin conditions. These studies suggest a co-evolutionary relationship between humans and their gut microbiome. Another study by Sharma et al. (2018) reported mannan and probiotic synergistic effects, where feces from buffalo fed with *Lactobacilli* and MOS exhibited an increased *Lactobacilli* and *Bifidobacterium* abundance and an *Escherichia coli* abatement compared to *Lactobacilli*-only fed buffalo [61].

Yeast mannan has potential to be used as an anti-bacterial agent. Especially, affinity of *E. coli* with *Saccharomyces boulardii* was reportedly higher than with *S. cerevisiae* [54]. *S. boulardii* mannan is a sponge-like structure that absorbs enteric pathogens, such as *Salmonella enterica* Typhimurium and *E. coli* O157, through mannan-specific adhesins [54]. Ofek and Beachey (1978) confirmed that piliated *E. coli* can be attached to epithelial cells by recognizing the receptor on the surface of epithelial cells. The *E. coli* attached to epithelial cells can be displaced from the epithelial cells with α-D-mannopyranoside of yeast [62]. In addition, Baurhoo et al. (2007) treated broilers with antibiotics or MOS feed [63]. Populations of beneficial bacteria, including *Bifidobacterium* and *Lactobacilli*, were elevated, and the *E. coli* population was lower in the ceca of broilers fed MOS, without boiler growth change between the two diets [63]. Moreover, Browne et al. (2019) reported that mannose treatment could reduce TNF-α secretion in *E. coli*-infected HT-29 cells, curtailing inflammation [64]. Mannan is a potential antibiotic replacement without any reported antibiotic resistance [65]. In a study by Smith et al. (2020), the yeast cell wall mannan-rich fraction reduced ampicillin-susceptible and -resistant *E. coli* proliferation [65]. Based on these studies, yeast cell wall mannan could be an alternative strategy to promote animal health without contributing to the problem of antibiotic resistance.

Mannan can also suppress mycotoxin adsorption [66]. In a study by Hafner et al. (2012), MOS protected rabbits against genotoxic T-2 toxins *Fusarium* produces. Rabbit lymphocytes exposed to T-2 toxin with MOS expressed a significantly lower genotoxic effect than those only exposed to T-2 toxins [67]. These results substantiate that the protective effects of MOS against fungal mycotoxin may be due to its binding and antioxidant properties in the gut.

3.2. Immune boosting properties

Yeast mannan activates immune cells, including macrophages, dendritic cells, T cells, and epithelial cells [68]. This activation is a cascade through N-linked α-mannose-specific recognition of C-type lectin receptors, such as dectin-2, mannose-binding lectin, mannose receptors, and dendritic cell receptors [69]. Numerous *in vitro* and *in vivo* studies have examined mannan's immune modulator capability [68,70]. Yeast cell wall mannan can interact with intestinal epithelial cells [71]. In animal model studies, *S. cerevisiae* mannan improves innate immunity by upregulating Sheep beta-defensin-1 (SBD-1) expression, an antimicrobial peptide secreted in ovine ruminal epithelial cells (OREC) [71]. The dectin-2 receptor on OREC recognizes *S. cerevisiae* mannan components and activates MAPK (MAP kinase) and the NFκB (Nuclear Factor kappa B) signaling pathway, promoting immune responses [71]. In another animal model, parasite-infected neonatal chicks were fed a diet supplemented with or without MOS. As a result, local mucosal IgA secretion was promoted and parasitic infections were reduced in the group supplemented diet with MOS, compared to the group

supplemented diet without MOS [72].

Furthermore, fish Nile tilapia (*Oreochromis niloticus*) provided with 0.2% and 0.1% mixture of β-glucan and MOS (Immunowall® dietary levels) for two months exhibited elevated white blood cell count, total protein, and globulin concentrations [73]. In addition, immune parameters such as antioxidant biomarkers (catalase and glutathione reductase), non-specific immune responses (phagocytic activity, phagocytic index, and lysozyme activity), and immune-related genes expressions (TNF-α and IL-1β) were high in the 0.2% diet group, reducing *Lactococcus gravieae* and *Aeromonas hydrophila* infection mortalities [73].

Candida albicans cell wall mannan also can be used as an immune modulator [74]. Mannoprotein from *C. albicans* stimulated pro-inflammatory cytokines and gene expressions related to M1 polarization, a pro-inflammatory state, and increased phagocytosis through the Akt signaling pathway within macrophages [74]. MOS predominantly targets monocyte lineage cells, including T and B lymphocytes and fibroblasts [75]. Furthermore, mannan can be utilized for DNA vaccine delivery via vaccination-conjugated mannan systems. Tang et al. (2009) demonstrated that the mannan-based system for delivering DNA vaccines to antigen-presenting cells could induce considerably enhanced immune responses in mice compared to naked DNA immunization, prefacing the molecular basis of immune-enhancing activity for mannan-based DNA vaccination [16]. Additionally, Vu-Quang et al. (2012) also used carboxylic mannan-coated iron oxide nanoparticles to target immune cells for *in vivo* lymph node-specific magnetic resonance imaging [17]. Mannan from Carboxylic mannan-coated iron oxide nanoparticles is recognized by immune cell receptors, preferentially promoting the uptake of the nanoparticles into immune cells [17]. Specifically, biocompatible self-assembled mannan nanogels were designed as a therapeutic or vaccine delivery platform for targeting mannose receptors expressed on antigen-presenting cell surfaces [76].

3.3. Antioxidant

Excessive oxidative stress is marked by an imbalance between free radical levels and the body's protection mechanisms [77]. Oxidative stress including reactive oxygen species (ROS) affects cellular proteins, membrane lipids, and DNA [78]. Lethal and irreversible cellular damage from oxidative stress can result in cancer, cardiovascular diseases, and diabetes [79,80]. Thus, antioxidants are a prominent touchstone in preventing and treating these diseases [81].

A study investigated the characteristics and antioxidant properties of mannan from *Saccharomyces cerevisiae*, emphasizing molecular weight differences [82]. Mannan was isolated via ethanol precipitation, resulting in fractions YM172 (172.90 kDa), YM87 (87.09 kDa), and YM54 (54.05 kDa). These mannans showed varied antioxidant activities in the DPPH assay, influenced by molecular weight. Notably, the smallest molecular weight fraction, YM54, exhibited the highest antioxidant effectiveness [82]. MOS is a promising antioxidant. Križková et al. (2001) examined antioxidant activity of yeast cell wall mannan *in vitro* [83]. *Candida utilis*, *S. cerevisiae,* and *C. albicans* mannan were isolated and scrutinized for antioxidant and anti-mutagenic activity, revealing various antioxidant and anti-mutagenic activities for each mannan [83]. *C. utilis* mannan reported the highest antioxidant and anti-mutagenic activity, signifying that mannan antioxidant activity mediates anti-mutagenic DNA protection [83]. Xiao et al. (2012) corroborated this finding, demonstrating that mannose can affect genes involved in nutrition metabolism, immunity, and cell cycle biological processes within broiler jejunum [84]. Furthermore, the authors verified that metabolic process genes are most affected and that genes related to antioxidants can be induced by MOS [84].

3.4. Other bioactive effects

Yeast cell wall mannan primarily exhibits improved immune responses, intestinal health, and antioxidant biological effects [23,85]. However, less-known effects include enhanced hyperlipidemia and triglycerides. For example, a study by Yoshida et al. (2009) investigated the structural features of mannan of *Kluyveromyces marxianus* YIT 8292 and its hypocholesterolemic activity. Mannan from *K. marxianus* YIT 8292 has shorter α-(1 → 2) linked side chains and lower phosphate content compared to mannan from *Saccharomyces cerevisiae*. The structural properties of mannan present in YIT 8292 strain showed better hypocholesterolemic activity in experiments on rats fed a high-cholesterol diet. *S. cerevisiae* mutant strain with similar mannan structural modifications as YIT 8292 strain also showed higher hypocholesterolemic activity, suggesting the importance of side chain length and phosphate content in mannan function for hypocholesterolemic activity [86]. Korolenko et al. (2018) observed that mannan from *C. albicans* improves hyperlipidemia. The authors extracted α-1,2-, α-1,3-, β-1,2-linked mannopyranose units for mannan A and only α-1,2-linked mannopyranose units for mannan B. In the acute lipemia mouse model, mannan A and B lowered LDL cholesterol, total cholesterol, and triglycerides in the blood and decreased hepatocyte lipid droplets. Especially, the structurally simpler mannan B exhibited more potent hypolipidemic effect [87]. In a recent study by T.A Korolenko et al. (2020), female mice were fed a high-cholesterol diet with or without 1% MOS for 14 weeks, revealing that MOS consumption inhibited the increased plasma cholesterol levels. In addition, the interaction between MOS and intestinal microflora increased beneficial bacteria and butyrate levels in feces [88].

Hashimoto et al. (1983) identified an acidic mannan yeast fraction with antitumor activity [89]. Feeding mice with yeast mannan acidic fraction, containing sugar (93.6%), nitrogen (1%), and phosphorus (0.6%), elevated the peritoneal cell proliferation and macrophage lysosomal activity. The authors suggested that this effect may be related to the nitrogen and phosphorus content present in the acidic mannan yeast fraction [89]. In another study, Galinari et al. (2017) reported that MOS from *K. marxianus* could inhibit the proliferation of tumor cells [90]. Tumor cell line, HEP-G2, and the non-tumor mouse fibroblast line, 3T3-L1 were treated with MOS at 0.5 mg/mL for 24 h. The result revealed that only the proliferation of HEP-G2 cell was inhibited, leaving the 3T3-L1 fibroblast cells unaffected [90].

Katrlík et al. (2022) developed a surface plasmon resonance (SPR) biosensor, which applies isolated mannan from *Candida dubliniensis* yeast to detect viral and bacterial pathogenesis by interacting with glycan molecules [15]. The SPR biosensor is a device

capable of monitoring viral and bacterial infections by using binding interactions between surface molecules. The SPR biosensor chip based on mannan detected 0.1 nM Concanavalin A, a type of lectin [15]. This result suggested that mannan-based SPR biosensor signify the immune response of lectins bound to pathogenic factors efficiently [15].

4. Yeast mannan production

4.1. Yeast mannan preparation

Mannan accounts for 20–50% of the cell wall, bound at a 90% mannose to 10% protein ratio [21,91]. Mannan preparation from yeast entails separation, extraction, and purification [23]. During the separation process, cells are physically disrupted, and extraction methods include alkali, acid, and enzymatic processes (Table 3). Purification deproteinizes polysaccharides is including the Sevag method, where proteins are denatured and precipitated by shaking with chloroform solution [92]. Also, an isoelectric method, where protein even with low solubility is denatured and precipitated at its isoelectric and boiling point, or chromatography method is used for mannan preparation [93].

Several studies have attempted to determine optimal mannan extraction conditions [85,93]. For example, Yang Liu et al. (2018) optimized mannan extraction from yeast cell walls by alkaline and deproteinization methods. The authors treated yeast cell wall with NaOH solution (0.5%, 1%, 3%), neutralized with HCl solution and then separated the supernatant. The supernatant was subjected to deproteinization using either the Sevag or isoelectric point methods. Mannan was then isolated by ethanol precipitation. As a result, the best yield of 18% mannan was achieved using 1% NaOH for 2 h at 100 °C, followed by purification with the isoelectric point method [93]. Huang (2008) attempted an alternative alkali mannoprotein extraction [94]. (1–3)-β-D-glucan is alkali-insoluble, whereas mannoprotein is alkali-soluble; thus, the authors extracted (1–3)-β-D-glucan using 6% NaOH at 60 °C over 4 h [94]. A spray dryer was used to dry the insoluble fraction (1–3)-β-D-glucan, achieving a 13.5% (1–3)-β-D-glucan yield. In contrast, mannoprotein extraction conditions were set to 1% NaOH at 100 °C for 2 h. Mannoproteins were precipitated using absolute ethanol, and residual proteins were purified through Sevag method. However, the extracted mannan yield was not reported [94].

Alternatively, François (2006) analyzed yeast cell wall polysaccharides through acid extraction methods based on sulfuric acid hydrolysis [95]. First, a bead beater disrupted *S. cerevisiae* for cell wall isolation, and polysaccharides were extracted through 72% H_2SO4 solution for 3 h at room temperature. Then, the acid solution was neutralized to a pH 6.0–8.0 before centrifugation [95]. Analysis of the solution's supernatant confirmed a 93.3 μg of yeast mannan per mg of *S. cerevisiae* cell mass cultured in YPD media [95]. Schiavone et al. (2014) also tested chemical methods for quantitative analysis of yeast cell wall mannan. A bead beater disrupted the *S. cerevisiae* BY4741 strain, and the cell wall fraction was hydrolyzed with 2 N H_2SO_4 at 100 °C for 4 h [96]. As a result, mannan from yeast cell wall accounts for 34.3 % of total cell wall polysaccharides [96].

An enzymatic method also was used to extract the cell wall mannan of yeast [97]. One study described a mild enzymatic method for the isolation of mannan from the cell walls of *S. cerevisiae* [97]. The process begins with enzymatic digestion, followed by mechanical disruption with glass beads. Using enzyme cocktails of protease, β-1,3 glucanase, and cellulase, cell wall components were digested to separate soluble mannan. Through chromatography, 61 mg of mannan was isolated from 250 mg of yeast cell walls [97]. Another study compared different techniques for extracting mannoproteins from yeast cell walls (YCW), including heat treatment, sodium dodecyl sulfate (SDS) extraction, and enzymatic treatment using Zymolyase [98]. The enzymatic method emerged as the most efficient, achieving the highest mannoprotein yield at 46.7%, thereby outperforming the other methods evaluated [98].

Table 3
Extraction methods for yeast mannan preparation.

Class	Strains	Mannan type	Extraction	Cell wall isolation	Treatment conditions	Purification	Reference
Yeast	*Saccharomyces cerevisiae*	Mannan	Acid	Bead beater	72% H_2SO_4; 20–25 °C; 3 h followed by 2 N H_2SO_4; 100 °C; 4 h	–	[95]
	Saccharomyces cerevisiae	Mannan	Acid	Bead beater	2 N H_2SO_4; 100 °C; 4 h	–	[96]
	Saccharomyces cerevisiae	Mannan	Alkali	–	1 % NaOH; 100 °C; 2 h	Column chromatography	[93]
	Saccharomyces cerevisiae	Mannan	Alkali	–	1 % NaOH; 100 °C; 2 h	Sevag method	[94]
	Saccharomyces boulardii	Mannan	Enzymatic	–	Zymolyase; 45 °C; agitation (200 rpm)	Affinity chromatography	[98]
	Saccharomyces boulardii	Mannan	Enzymatic	Ultrasound	ultrasound 20 kHz and lyticase; 2 h	–	[173]
	Schizosaccharomyces pombe	Glucomannan	Acid	Glass beads in a homogenizer	1 N HCl; 100 °C; 1 h	–	[174]
	Kluyveromyces marxianus	Mannan	Alkali	–	3% NaOH; 80 °C; 6 h	–	[90]
	Candida albicans	Mannan	Alkali	–	3% NaOH	Ultrafiltration and anion-exchange chromatography	[74]

5. Mannan overproduction strategy

5.1. Wild-type yeast screening and selection

Proliferation determines yeast physiological and morphological changes, such as cell wall thickening [99]. For instance, Valentín et al. (1987) confirmed yeast growth phase affects cell wall composition [100]. Cell wall mannan changes between exponential to stationary phases, expressing the highest concentration during the late exponential growth phase [100]. Hamada et al. (1984) investigated the relationship between cell wall mannan and growth media NaCl concentrations in *Saccharomyces rouxii* [101]. *S. rouxii* mannan contents were slightly diminished in a growth medium with 15% NaCl compared to *S. rouxii* grown without NaCl [101]. Another study reported that environmental stress, such as high temperature and osmolality, can release cell wall mannan into the growth medium [102].

Yeast mannan structure contents also differ by strain. *Candida auris* structures contain more mannose residue, so it has a higher immunoglobulin G (IgG) affinity than *C. albicans* [103]. Yoshida et al. (2009) reported that *Kluyveromyces marxianus* YIT 8292 expresses more hypocholesterolemic activity than other yeast strains, such as *S. cerevisiae* [86]. The authors conclude this difference is because *K. marxianus* YIT 8292 cell wall mannan have shorter α-(1,2)-linked oligomannosyl side chains and lower phosphate contents than *S. cerevisiae*, suggesting that these characteristics affect yeast's biological functions [86]. Cell wall integrity and compositions are influenced by the cell cycle phase and culture conditions such as pH, aeration, and temperature [19,104]. One study was conducted to observe the mannan content of yeast cell walls over the culture time [45]. The *S. cerevisiae* M21 and *S. cerevisiae* M54 strains were cultured at 28 °C using barley wort extract medium. The mannan content of the cell wall increased significantly at the early stationary phase of the culture, which occurred at 9 h, compared to the initial cultivation. The mannan content accounted for 16.66% and 10.96% of the dry weight of the cell wall in strains M21 and M54, respectively. These results suggested that both strain and incubation period affect the mannan content of the cell wall [45].

5.2. Saccharomyces boulardii and other yeast mannan

Different yeast species from baker's *S. cerevisiae* yeast to pathogenic *C. albicans* contain mannan with variation in the structural composition and arrangement of mannan molecules [105]. However, even mannan from various yeasts differing in size and degree of branching express similar reactivity [106], such as *S. cerevisiae*, *S. boulardii*, *Pichia kudriavzevii, P. fermentans*, *K. marxianus*, *K. lactis*, *Debaryomyces hansenii*, *Torulaspora delbrueckii*, and *Yarrowia lipolytica* [107].

S. boulardii is the only commercial probiotic yeast, and its therapeutic properties have been thoroughly evidenced in over 80 randomized clinical trials based on Laboratoires Biocodex's *S. boulardii* CNCM I-745 strain (or *S. boulardii* Hansen CBS 5926) [108]. *S. boulardii* is isolated from fruit, lychee, and mangosteen skins [109], generally regarded as safe by FDA, and used in gastrointestinal dysbiosis treatments [110,111]. It has a high growth temperature (37 °C) and tolerance to acidic conditions (pH 2), providing improved viability in the gastric environment [112].

Despite their genomic similarities, *S. boulardii* ATCC MYA-796 has a higher cell wall mannan content [19]. Recent quantitative analysis confirmed that cell wall fraction of *S. boulardii* has higher mannan contents than that of *S. cerevisiae* S288C [19]. The ability of *S. boulardii* to bind bacterial pathogens is correlated with mannose residue presence [54] N- linked and O- linked oligosaccharide compositions in cell wall glycoproteins vary, with *S. cerevisiae* and *C. albicans* indicating high mannan structures and *Schizosaccharomyces pombe* having galactomannan [113]. Schweigkofler et al. (2002) analyzed and determined three monosaccharide patterns in disparate yeast cell walls: (a) the glucose-mannose type; (b) the glucose-mannose-galactose type, and (c) the glucose-mannose-galactose-rhamnose type [114]. The glucose-mannose type predominated, prevalent in 51 species of the genera *Saccharomyces*, *Pichia*, *Candida*, *Debaryomyces*, and *Kluyveromyces*, with substantially different mannose proportions ranging from 22% to 75%. The glucose-mannose-galactose type was observed in 26 *Pichia*, *Candida*, *Arxula*, *Debaryomyces*, *Sz. Pombe,* and *Yarrowia* genera strains [114].

Among fungal polysaccharides, the negatively charged phosphate groups within phosphomannan structures garner particular interest. For example, *Pichia holstii* contains a highly branched extracellular phosphomannan core bearing oligosaccharide sidechains attached via phosphodiester bonds [115]. The phosphomannan core effectively inhibits *in vivo* lymphocyte migration [116]. Furthermore, the oligosaccharide phosphate fraction derived from this polysaccharide is used to manufacture PI-88, a phosphomanno-pentaose sulfate with several medically significant properties such as inhibitor of tumor growth and metastasis [117].

Kuraishia capsulata contrasts many yeast species by accumulating polyphosphates and phosphomannan, which contain a substantial portion of inorganic phosphates [118]. According to Ustyuzhanina et al. (2018), linear mannan and branched phosphomannan production in *Kuraishia capsulate* are predicated by phosphate concentration within the culture medium. Only mannan was obtained when phosphate was removed, while a KH_2PO_4 excess produced phosphomannan [119]. *Kluyveromyces marxianus* CCT7735 cell wall α-D mannan fraction signified anti-proliferative and improved antioxidant activity, evidenced by the waning power potential, metal chelating, and hydroxyl radical scavenging activities [120].

Galactomannans are integrated into the cell wall structures of other fungal species, such as *Schizosaccharomyces Pombe* and *Aspergillus fumigatus* [121]. Agboola et al. (2021) reported that mannans constituted 8.7%, 11.5%, and 16.7% of autolyzed yeasts cell walls for *Blastobotrys adeninivorans*, *Cyberlindnera jadinii*, and *Wickerhamomyces anomalus*, respectively [122]. Cell wall mannan isolated and purified from the human pathogenic yeast *C. dubliniensis* CCY 29-177-1 constituted D-mannose and traces of D-glucose residues [123]. Its backbone exhibited α-1,6-linked mannose residues next to side mannose residues as single stubs and side oligosaccharide chains of d. p. 2–7 primarily as tri-, di-, and tetramer forms. Long side chains, such as penta, hexa, and heptamers, were less

common and α-1,2-linked and α-1,3-linked mannose residues formed these side chains [123].

5.3. Random and directed mutation

High mannan-producing mutant yeast strains can be selected after random mutation by physical, biological, and chemical mutagens (Fig. 3). Ha et al. (2006) attempted to generate an *S. cerevisiae* YPH499 cell wall mutant strain using ultraviolet (UV) irradiation and laminarinase (*endo*-β-(1,3)-D-glucanase) enzyme treatment. The resulting K48L3 strain had a 2255 μg mannoprotein from 100 mg dry weight of yeast, an approximately 3-fold improvement [124]. Furthermore, the mutated strain conveyed enhanced immune functions compared to the wild-type. Similarly, Quirós et al. (2010) harnessed UV mutation to establish the yeast with the most mannoproteins. The *S. cerevisiae* BY4741 strain, grown in a k9 toxin-containing YPD medium, was irradiated under a 254 nm UV lamp to separate mannan from the yeast cell wall and to produce mannan-rich wine. The resulting strain released 70% more mannan than the wild-type strain [125]. Ribas et al. (1991) mutated the cell wall mannan structure of *Schizosacharomyces pombe* using ethylmethane sulfonate (EMS) [126]. The cell wall composition was extracted through alkali extraction methods for evaluation. The mutant strain exhibited a cell wall mannan content that was 4-fold lower than that of the wild-type. This suggested that the EMS-induced mutation specifically affected the synthesis or incorporation of galactomannan in the cell wall of the mutant *S. pombe* strain. No significant changes were observed in other cell wall components, such as β-glucan and chitin [126]. Lai et al. (2020) used ethylmethane sulfonate to mutate *S. cerevisiae* BCRC 21685. The mutant strain for overproducing mannoproteins was selected using killer-9 toxin-containing YPD media [127]. Strains that showed significant resistance to the killer-9 toxin were compared for mannoprotein content. The mutant strain had 386.8 mg mannoprotein per g dry cell mass, higher than the wild-type containing 298 mg mannoprotein per g dry cell mass [127].

5.4. Rational engineering of mannan biosynthetic pathway

Yeast cell wall biosynthetic pathway is a complex process that involves approximately 1200 genes [128] related to glycolysis, GDP-mannose synthesis, endoplasmic reticulum (ER), and Golgi apparatus (Fig. 4). Mannoprotein exists in combination with branched glucan [129]. Mannoproteins have two glycosylation types (*N*-glycosylation and *O*-mannosylation) synthesized from guanosine diphosphate (GDP)-mannose [130,131]. GDP-mannose is synthesized from upper glycolysis intermediates, fructose 6-phosphate [19]. This synthetic process involves several enzymatic steps. First, Fructose 6-phosphate is transformed into mannose 6-phosphate by mannose 6-phosphate isomerase (*PMI40*). Mannose 6-phosphate is converted to mannose 1-phosphate, catalyzed by phosphomannomutase (*SEC53*). Lastly, mannose 1-phosphate is biosynthesized to GDP-mannose by GDP-mannose pyrophosphorylase (*PSA1*) [19]. The synthesized GDP-mannose moves to the ER and serves as a sugar donor [132].

A highly glycosylated mannan layer is initiated by mannosyltransferases in the ER [129]. This N-glycosylation process produces a

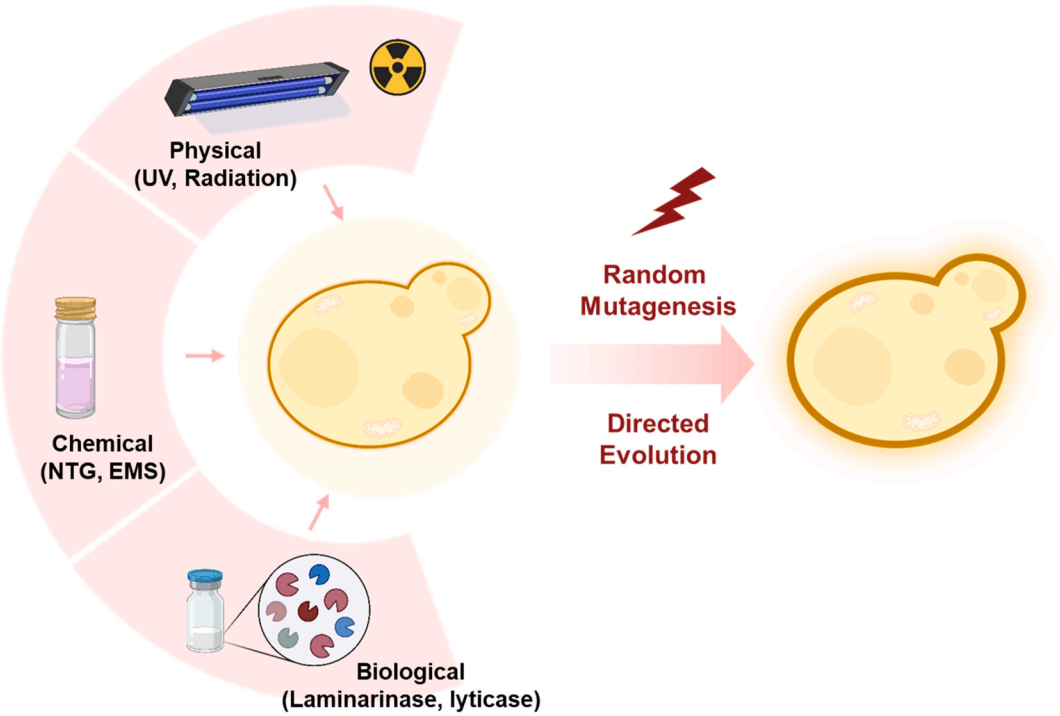

Fig. 3. Method for construction of high mannan-producing mutant yeast. This figure was created with BioRender (https://biorender.com/).

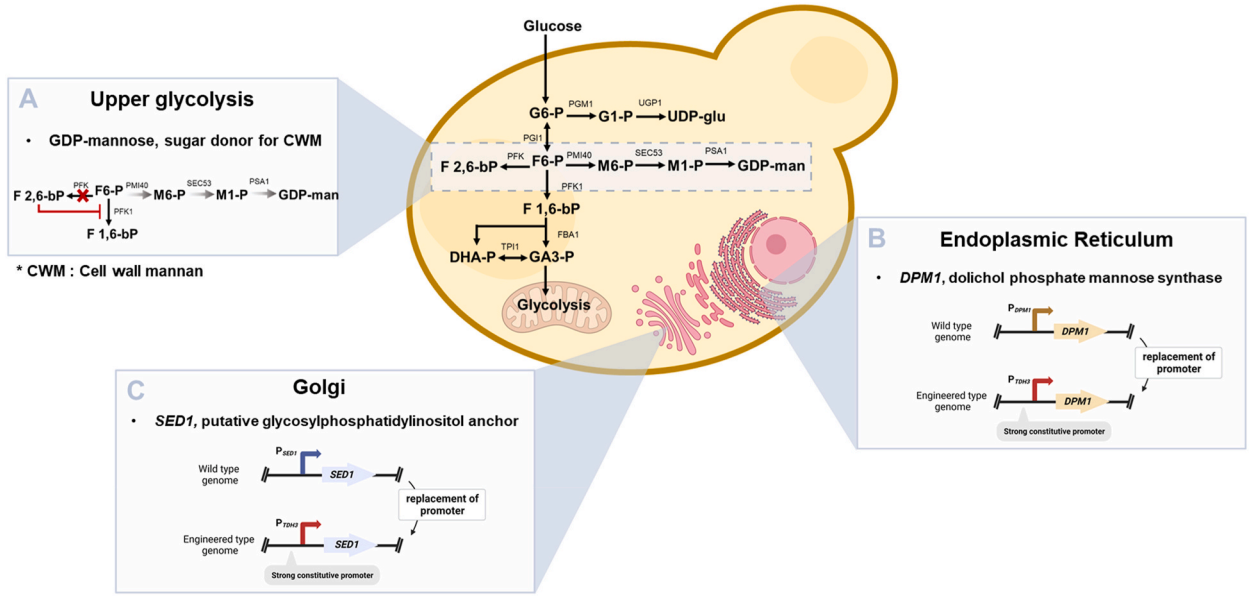

Fig. 4. Biosynthetic mannan pathways and strategies for overproduction.This figure was created with BioRender (https://biorender.com/).

different structure combination. In particular, N-glycosylation displays α-1,6 linked backbone and α-1,2, α-1,3-linked side chains [129]. In N-glycosylation, GDP-mannose combines with Dol-pp-$GlcNAc_2$ present in the ER catalyzed by mannoslytransferase resulting in the formation of Dol-pp-$GlcNAc_2$-Man [130]. Dol-pp-$GlcNAc_2$-Man is sequentially added a mannose, leading Dol-pp-GlcNAc2-Man_9 [132]. Dol-pp-$GlcNAc_2$-Man_9 is glycosylated by transfer of three glycosyl units from UDP glucose, generating $Glc_3Man_9GlNAc_2$ [132]. $Glc_3Man_9GlNAc_2$ transfer to asparagine (Asn) residue in protein sequence by oligosaccharyl transferase complex [130]. $Glc_3Man_9GlNAc_2$ combined with asparagin is then cutting by glucosidases and mannosidase to remove three glucose (Glc) and one mannose (Man) residue, generating a $Man_8GlcNAc_2$ [130]. $Man_8GlcNAc_2$ combined with asparagin is extended by the action of Golgi mannosyltransferases [130]. O-mannosylation has a short mannose chain, and the first step is initiated in the ER [130]. GDP-mannose is combined with dolichol phosphate by Dol-P-Man synthase (*DPM1*) to form dolichyl-phosphate-mannose. In dolichyl-phosphate-mannose, mannose is transferred to serine or threonine residues by O-mannosyltransferases [130]. Subsequent steps occur in the Golgi [133].

Glycosylation is modified further in the Golgi [133]. N-glycosylated proteins, transported from the ER to the Golgi are combined with α-1,6-mannose of up to 50 residues is synthesized by mannose polymerase complexes in Golgi [134]. Mannosyltransferase encoded by the *MNN1* family and *KTR* family adds α −1,2 branched chains to the α −1,6-mannose backbone [135,136]. Subsequently, outer chains are terminated by the addition of an α −1,3-linked mannose by Mannosyltransferase [130]. In O-mannosylated proteins, the α-1,2 linkages are catalyzed by mannosyltransferases encoded by *KTR* family members. Then the terminal α −1,3 links is carried out by mannosyltransferases encoded by *MNN1* family members [137]. Yeast glycoproteins are terminated through mannosylation in the Golgi; therefore, cytoplasmic mannose transport to the Golgi apparatus is crucial. Rational engineering of the pathways related to yeast cell wall synthetic can generate the engineered yeast strains which have higher MOS and mannoprotein contents in cell wall.

Kwak et al. (2022) enhanced *S. boulardii* (ATCC MYA-796) probiotic properties by increasing the cell wall's mannan content. This mannan biosynthesis was enhanced by increasing sugar-phosphate intermediate availabilities in upper glycolysis. First, the obstructive pathway that produces GDP-mannose, the precursors for cell wall oligosaccharides, was blocked using the CRISPR Cas9 system to obtain the Sb-p strain and delete the *PFK26* and *PFK27* open reading frames that encode 6-phosphofructo-2-kinase isozymes. However, this strategy only slightly increased cell wall mannan content. Thus, the authors overexpress *PMI40*, *SEC53,* and *PSA1* encoding mannose 6-phosphate isomerase, phosphomannomutase, and GDP-mannose pyrophosphorylase incorporated the strong constitutive promoter P_{TDH3} to enhance the GDP-mannose pathway and obtain the SbM2-p strain, which is 5.8-fold higher than the wild-type (0.37 mg/g cell) [19].

In addition, mannoproteins and the transport mechanisms in the ER and Golgi were manipulated to intensify cell wall mannan content. The gene of *SED1* and *DPM1* encoding a cell wall mannoprotein carrying multiple N-glycosylation sites and dolichol phosphate mannose synthase was overexpressed under the control of P_{TDH3}, obtaining the strain SbM2SD-p, exhibited 12.7% high cell wall mannan content than that of SbM2-p. Finally, it is confirmed that the strain SbM2SD-p can inhibit the growth of harmful microorganisms such as *Clostridioides difficile.* CWO-engineering process was accomplished through CRISPR-Cas9 genome editing without heterogeneous genetic elements, resulting in non-transgenic *S. boulardii* strains with enhanced CWO-derived benefits [19]. These results showed that the rational engineering of yeast such as *S. boulardii* can generate high mannan-producing strain.

In Golgi, there are many potential genetic engineering targets which can alter mannan contents in cell wall of yeast such as *S. cerevisiae*. Conde et al. (2003) investigated yeast cell wall mannan-related genes through 622 deletion strains generated during the

EUROFAN B0 project. Two genes, *MNN4* and *MNN6* located in Golgi, were directly involved in modifying yeast mannan's phosphate content [138]. In another study, the *S. cerevisiae* mutant strain was created by mutation of *VRG4* functioning, a GDP-mannose transporter (GMT). the mutant strain harboring the *VRG4* mutation caused a cell wall defect [139]. This result suggests that GDP-mannose transportation by the *VRG4* in Golgi is crucial for cell wall biosynthesis [139]. Increased mannoprotein contents can result in high mannan contents. The *Sc*Mnn9 which is glycosyltransferase in *S. cerevisiae* is vital for supplementing mannosylated proteins that shape yeast cell wall mannan [140]. Furthermore, High mannose content can also result from increased N-glycosylated cell wall proteins, such as *Sc*Ccw12, *Ca*Pga59, and CaPga62. Their corresponding genes each have an exceptionally high codon adaptation index (0.87, 0.95, and 0.91, respectively; *Saccharomyces* Genome Database and *Candida* Genome Database), thus encoding highly abundant proteins [141].

In ER, several target genes which can help to increase mannoprotein levels in *S. cerevisiae* were found. Schiavone et al. (2015) conducted transcriptomic analysis between L71 and L69, two industrial *S. cerevisiae* strains with different cell wall compositions [142]. Strain L71 had higher chitin and β-(1,6)-glucan levels than L69, yet L69 had approximately 20–25% higher mannan levels than L71 [142]. Transcriptome analysis distinguished 392 disparate genes between L71 and L69 strains [142]. Notably, the L69 strain indicated higher mannoprotein-encoding gene expressions than L71, including FLO_{11}-encoded flocculin (a GPI-anchored cell surface glycoprotein) and *YHR213w*-encoded putative flocculin [142]. The authors postulate that flocculin expression levels strongly correlate with mannan quantities in the cell wall [142]. These studies provide the potential gene targets for genetic perturbation which may increase contents of cell wall mannan and mannoproteins to generate high mannan-producing yeast strains.

5.5. Heterologous mannan production by engineered yeast

Genetic engineering allows to generate recombinant yeast strains which produce heterologous mannan such as plant mannan. Voiniciuc et al. (2019) manipulated *Pichia pastoris* to produce a plant mannan composed of mannose and glucose linked by β-(1–4). The cellulose synthase-like A (CSLA) coding sequence from *Amorphophallus konjac* was integrated into *P. pastoris* genome to produce plant mannan in the cell wall [32]. Additionally, the authors introduced a mannan-synthesis-related (MSR) coding sequence from *Arabidopsis thaliana* into *P. pastoris* genome, resulting in increased plant mannan contents in the cell wall by about 70%. Thus, it was confirmed that the CSLA and MSR could affect plant mannan production in *P. pastoris* [32].

To further increase plant mannan production in *P. pastoris*, Robert et al. (2021) constructed chimeric CSLA proteins that assembled β-1,4-linked mannan in quantities superior to native enzymes while minimizing yeast growth burden by swapping plant mannan synthase domains [143]. Moreover, plant mannan yield and glucose incorporation could be further increased by co-expressing chimeric CSLA proteins with a MSR co-factor [143]. These studies demonstrate strain engineering strategy to produce heterologous mannan in yeast as a host strain.

6. Conclusions

This review comprehensively elucidates practical and theoretical recommendations for advancing yeast cell wall polysaccharide research, specifically for mannan. Yeast cell wall has inner layer comprising β-1,3-glucan and chitin, accounting for about 50–60% of the cell wall dry weight and outer layer emanating mannan from the cell surface, account for approximately 30–50% of cell wall mass. Oligosaccharides derived from yeast cell wall are used in many commercial applications. Especially, current scientific knowledge has established yeast cell wall mannan as a promising element in the functional foods and nutraceuticals sector.

Additionally, long mannan chains are hydrolyzed into MOS. Due to their bioactivity, yeast-derived MOS are utilized as prebiotics in animal husbandry and nutritional supplements. Several studies have reported that beneficial health effects of yeast cell wall mannan, including gut health, immune regulation, antioxidant properties, reduction of hyperlipidemia, anti-cancer properties, and vaccine enhancer.

On the other hand, Mannan of the yeast cell wall can be extracted by various methods which are alkali, acid, and enzymatic extraction processes. Depending on the preparation method of mannan, the amount of mannan recovered from the cell wall, as well as its molecular weight or structure of mannan can vary. Enzymatic extraction processes are known to be particularly suitable for mannoprotein extraction due to their simplicity. Employing a mixture of several enzymes can increase the efficiency of the enzymatic extraction process. Alkaline extraction methods and acid hydrolysis methods can also yield significant results, such as higher yield and improved purity. However, conditions such as concentration and temperature must be carefully optimized, and require a subsequent neutralization step. Ultimately, the choice of extraction method depends on the desired purity, yield, and intended use of the extracted mannan or mannoprotein, considering the variation in mannan recovery and molecular characteristics.

Additionally, yeast cell wall mannan contents vary depending on the strains and culture conditions including high temperature and osmolality. Mannan from various yeasts differ in size and degree of branching, which also affects the functionality of mannan. Mannan structure includes α-(1 → 6)-linked main chains and branches with α-(1 → 2), α-(1 → 3), or phosphodiester bridges. Yeast species, both pathogenic and non-pathogenic, produce mannan with unique structural features. For example, mannan of *Candida* genus has long side chains, while mannan of *Pichia* genus is known for their highly branched phosphomannan cores. These features influence solubility, body interactions, and immune response impacts. The side chains' length and branching degree are crucial for cell recognition and binding, affecting immune responses. However, mannan structure diversity complicates its extraction and characterization, affecting bioactivity and necessitating extensive testing for specific uses. Using mannan from pathogenic yeasts in food or medicine raises safety and regulatory concerns, requiring thorough approval. Despite challenges, mannan from various yeasts offer potential benefits, contingent on resolving extraction, bioactivity, and safety issues.

Selecting yeast strains with a highly established mannan content potential is crucial, as are strain engineering strategies for enhancing mannan syntheses, such as mutagenesis and metabolic engineering. Several studies offer potential targets for genetic engineering, which could enhance the contents of cell wall mannan and mannoproteins, leading to the creation of yeast strains that produce high levels of mannan. The development of yeast strains that produce high levels of mannan based on such advanced biotechnology will explore new possibilities for efficient mannan production and improve commercial use in various fields such as food and medicine. This review delineated yeast cell wall mannan components, structural features, biological activities, and underlying molecular mechanisms. Moreover, strategies to alter yeast strains for mannan overproduction were emphasized and discussed. This review also provided a scientific basis for yeast cell wall mannan research and industrial applications.

CRediT authorship contribution statement

Kwang-Rim Baek: Writing – original draft, Visualization, Investigation. **Sudha Rani Ramakrishnan:** Writing – original draft, Visualization, Investigation. **Soo-Jung Kim:** Writing – review & editing, Supervision. **Seung-Oh Seo:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] M. Parapouli, A. Vasileiadis, A.-S. Afendra, E.J.A.m. Hatziloukas, *Saccharomyces cerevisiae* and its industrial applications 6 (1) (2020) 1.
[2] I. Sundh, P. Melin, Safety and regulation of yeasts used for biocontrol or biopreservation in the, food or feed chain 99 (2011) 113–119.
[3] de Oliveira A.H., Alcaraz-Espinoza J.J., da Costa M.M., Nascimento M.L.F., Swager T.M., de Oliveira H.P., Improvement of Baker’s Yeast-Based Fuel Cell Power Output by Electrodes and Proton Exchange Membrane Modification,105 (2019): 110082.
[4] P. Srinivasan, C.D.J.N. Smolke, Biosynthesis of medicinal tropane alkaloids in yeast 585 (7826) (2020) 614–619.
[5] D. Liu, L. Ding, J. Sun, N. Boussetta, E.J. Vorobiev, Yeast cell disruption strategies for recovery of intracellular bio-active compounds—A review 36 (2016) 181–192.
[6] F.M. Klis, P. Mol, K. Hellingwerf, S. Brul, Dynamics of cell wall structure in *Saccharomyces cerevisiae* 26 (3) (2002) 239–256.
[7] A. Bzducha-Wróbel, S. Błażejak, M. Kieliszek, K. Pobiega, K. Falana, M. Janowicz, Modification of the Cell Wall Structure of *Saccharomyces cerevisiae* Strains during Cultivation on Waste Potato Juice Water and Glycerol towards Biosynthesis of Functional Polysaccharides, vol. 281, 2018, pp. 1–10.
[8] R. Bastos, P.G. Oliveira, V.M. Gaspar, J.F. Mano, M.A. Coimbra, E. Coelho, Brewer’s Yeast Polysaccharides—A Review of Their Exquisite Structural Features and Biomedical Applications, 277, 2022 118826.
[9] F. Freitas, C. Roca, M.A. Reis, Fungi as sources of polysaccharides for pharmaceutical and biomedical applications 3 (2015) 61–104.
[10] FactsandFactors, Specialty Yeast Market: by Type (Yeast Extract, Yeast Beta Glucan, Yeast Autolysates, Hydrolyzed Yeast, and Others), by Species (*Saccharomyces Cerevisiae*, *Pichia Pastoris*, *Kluyveromyces*, and Others), by Application (Feed, Food, Biofuels, Beverages, and Others), and by Regions – Global & Regional Industry Perspective, Comprehensive Analysis, and Forecast 2021 – 2026, Facts and Factors, 2021.
[11] R. Rakowska, A. Sadowska, E. Dybkowska, F. Swiderski, Spent yeast as natural source of functional food additives 68 (2) (2017).
[12] S. Brugman, W. Ikeda-Ohtsubo, S. Braber, G. Folkerts, C.M. Pieterse, P. Bakker, A comparative review on microbiota manipulation: lessons from fish, plants, livestock, and human research 5 (2018) 80.
[13] A. Al-Manhel, A.K. Niamah, Mannan extract from *Saccharomyces cerevisiae* used as prebiotic in bio-yogurt production from buffalo, milk 24 (5) (2017) 2259–2264.
[14] R. Tanihiro, K. Sakano, S. Oba, C. Nakamura, K. Ohki, T. Hirota, H. Sugiyama, S. Ebihara, Y.J.N. Nakamura, Effects of yeast mannan which promotes beneficial Bacteroides on the intestinal environment and skin condition: a randomized, double-blind, placebo-controlled study 12 (12) (2020) 3673.
[15] J. Katrlík, A. Holazová, I. Medovarská, I. Seilerová, P. Gemeiner, S.J.S. Bystrický, A.B. Chemical, SPR biosensor chip based on mannan isolated from, *Candida dubliniensis* yeasts applied in immunization effectiveness testing 350 (2022) 130883.
[16] C.K. Tang, K.-C. Sheng, S.E. Esparon, O. Proudfoot, V. Apostolopoulos, G.A. Pietersz, Molecular basis of improved immunogenicity in DNA vaccination mediated by a mannan based carrier 30 (7) (2009) 1389–1400.
[17] H. Vu-Quang, M. Muthiah, Y.-K. Kim, C.-S. Cho, R. Namgung, W.J. Kim, J.H. Rhee, S.H. Kang, S.Y. Jun, Y.-J. Choi, Carboxylic mannan-coated iron oxide nanoparticles targeted to immune cells for lymph node-specific MRI in vivo 88 (2) (2012) 780–788.
[18] I. Farinha, S. Baptista, M.A. Reis, F.J.L. Freitas, Influence of Dissolved Oxygen Level on Chitin–Glucan Complex and Mannans Production by the Yeast *Pichia pastoris* 12 (2) (2022) 161.
[19] S. Kwak, S.J. Robinson, J.W. Lee, H. Lim, C.L. Wallace, Y.-S. Jin, Dissection and Enhancement of Prebiotic Properties of Yeast Cell Wall Oligosaccharides through Metabolic Engineering, vol. 282, 2022 121379.
[20] R.S. Yehia, A.M. Saleh, M.B. Ismail, S. Al-Quraishy, O. Al-Amri, R. Abdel-Gaber, Isolation and characterization of anti-proliferative and anti-oxidative mannan from *Saccharomyces cerevisiae* 34 (2) (2022) 101774.
[21] F.M. Klis, A. Boorsma, P.W. De Groot, Cell wall construction in *Saccharomyces cerevisiae* 23 (3) (2006) 185–202.
[22] D.J. Manners, A.J. Masson, J.C. Patterson, The structure of a β-(1→ 3)-D-glucan from yeast cell walls 135 (1) (1973) 19–30.
[23] Y. Liu, Q. Wu, X. Wu, S.A. Algharib, F. Gong, J. Hu, W. Luo, M. Zhou, Y. Pan, Y. Yan, Structure, preparation, modification, and bioactivities of β-glucan and mannan from yeast cell wall: a review, Int. J. Biol. Macromol. 173 (2021) 445–456.
[24] Y. Liu, Q. Wu, X. Wu, S.A. Algharib, F. Gong, J. Hu, W. Luo, M. Zhou, Y. Pan, Y. Yan, Y. Wang, Structure, preparation, modification, and bioactivities of β-glucan and mannan from yeast cell wall: a review, Int. J. Biol. Macromol. 173 (2021) 445–456.
[25] E. Cabib, D.-H. Roh, M. Schmidt, L.B. Crotti, A.J. Varma, The yeast cell wall and septum as paradigms of cell growth and morphogenesis 276 (23) (2001) 19679–19682.

[26] Z. Holan, V. Pokorný, K. Beran, A. Gemperle, Z. Tuzar, J.J.A.o.M. Baldrián, The glucan-chitin complex in *Saccharomyces cerevisiae*: Precise location of chitin and glucan in bud scar and their physico-chemical characterization 130 (1981) 312–318.
[27] G.L. Utama, L. Oktaviani, R.L. Balia, T. Rialita, Potential Application of Yeast Cell Wall Biopolymers as Probiotic Encapsulants 15 (16) (2023) 3481.
[28] H. Zlotnik, M.P. Fernandez, B. Bowers, E.J. Cabib, *Saccharomyces cerevisiae* mannoproteins form an external cell wall layer that determines wall porosity 159 (3) (1984) 1018–1026.
[29] Y. Jigami, T. Odani, Mannosylphosphate transfer to yeast mannan, Biochim. Biophys. Acta Gen. Subj. 1426 (2) (1999) 335–345.
[30] P.J.G. Orlean, Architecture and biosynthesis of the *Saccharomyces cerevisiae* cell wall 192 (3) (2012) 775–818.
[31] L. Moreira, E.X.F. Filho, An overview of mannan structure and mannan-degrading enzyme systems 79 (2008) 165–178.
[32] C. Voiniciuc, M. Dama, N. Gawenda, F. Stritt, M. Pauly, Mechanistic insights from plant heteromannan synthesis in yeast 116 (2) (2019) 522–527.
[33] T.L.D. Françoise, H. Routier, Richard D. Cummings, Marku. Aebi, in: Essentials of Glycobiology [Internet], 4th edition, 2022.
[34] J.-P. Joseleau, S. Pérez, The Plant Cell Walls: Complex Polysaccharide Nano-Composites, Glycopedia, 2016.
[35] A. Sudiana, H. Kuswendi, V. Dewi, R.J.J.A.H.P. Balia, The potential of ß-glucan from *Saccharomyces cerevisiae* cell wall as anti-cholesterol 9 (1) (2022) 72–77.
[36] F. Zhu, B. Du, A critical review on production and industrial applications of beta-glucans 52 (2016) 275–288.
[37] M. Peltzer, J.F. Delgado, A.G. Salvay, J.R. Wagner, β-Glucan, a promising polysaccharide for bio-based films developments for food contact materials and medical applications 22 (12) (2018) 1249–1254.
[38] R.E. Sabioni, F.S. Zanuzzo, J.E.P. Cyrino, Immunomodulation of Juvenile Pacu, Piaractus Mesopotamicus, by Different β (1-3)(1-6)-D Glucan Products, vol. 62, 2019.
[39] S.S. Ferreira, C.P. Passos, P. Madureira, M. Vilanova, M.A. Coimbra, Structure–function relationships of immunostimulatory polysaccharides: a review, Carbohydr. Polym. 132 (2015) 378–396.
[40] P.R. Mary, K.V.H. Prashanth, P. Vasu, M. Kapoor, Structural diversity and prebiotic potential of short chain β-manno-oligosaccharides generated from guar gum by endo-β-mannanase (ManB-1601), Carbohydr. Res. 486 (2019) 107822.
[41] S.Y. Yildiz, E.T. Oner, Mannan as a promising bioactive material for drug nanocarrier systems 9 (2014) 311–342.
[42] A. Hajhosseini, D. Doroud, A. Sharifan, Z. Eftekhari, Optimizing growth conditions of *Kluyveromyces marxianus* for Mannan production as a bioemulsifier 7 (2) (2020) 115–126.
[43] A.D. Pashov, B. Monzavi-Karbassi, T. Kieber-Emmons, Glycan mediated immune responses to tumor cells 7 (sup1) (2011) 156–165.
[44] R.J. Mrsny, Oral drug delivery research in Europe 161 (2) (2012) 247–253.
[45] R. Harbah, T. Meledina, D. Manshin, A. Morozov, The influence of cultivation conditions and strain of yeast on mannan polysaccharide content in cells, in: , 2022 30032.
[46] U.K. Jana, R.K. Suryawanshi, B.P. Prajapati, N. Kango, Prebiotic mannooligosaccharides: Synthesis, characterization and bioactive properties 342 (2021) 128328.
[47] R. Jha, J.M. Fouhse, U.P. Tiwari, L. Li, B.P. Willing, Dietary fiber and intestinal health of monogastric animals 6 (2019) 48.
[48] S.C.J.B.m. Bischoff, Gut health: A new objective in medicine? 9 (2011) 1–14.
[49] M. Chen, G. Ruan, L. Chen, S. Ying, G. Li, F. Xu, Z. Xiao, Y. Tian, L. Lv, Y. Ping, Y. Cheng, Y. Wei, Neurotransmitter and intestinal interactions: focus on the microbiota-gut-brain axis in irritable bowel syndrome, Front. Endocrinol. 13 (2022) 817100.
[50] M.L.Y. Wan, K.H. Ling, H. El-Nezami, M.F. Wang, Influence of functional food components on gut health, Crit. Rev. Food Sci. Nutr. 59 (12) (2019) 1927–1936.
[51] F. Cuskin, E.C. Lowe, M.J. Temple, Y. Zhu, E. Cameron, N.A. Pudlo, N.T. Porter, K. Urs, A.J. Thompson, A. Cartmell, A. Rogowski, B.S. Hamilton, R. Chen, T. J. Tolbert, K. Piens, D. Bracke, W. Vervecken, Z. Hakki, G. Speciale, J.L. Munōz-Munōz, A. Day, M.J. Peña, R. McLean, M.D. Suits, A.B. Boraston, T. Atherly, C. J. Ziemer, S.J. Williams, G.J. Davies, D.W. Abbott, E.C. Martens, H.J. Gilbert, Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism, Nature 517 (7533) (2015) 165–169.
[52] P. Spring, C. Wenk, A. Connolly, A. Kiers, A review of 733 published trials on Bio-Mos®, a mannan oligosaccharide, and Actigen®, a second generation mannose rich fraction, on farm and companion animals, Journal of Applied Animal Nutrition 3 (2015) e8.
[53] S. Oba, T. Sunagawa, R. Tanihiro, K. Awashima, H. Sugiyama, T. Odani, Y. Nakamura, A. Kondo, D. Sasaki, K. Sasaki, Prebiotic effects of yeast mannan, which selectively promotes Bacteroides thetaiotaomicron and Bacteroides ovatus in a human colonic microbiota model, Sci. Rep. 10 (1) (2020) 17351.
[54] B.R. Gedek, Adherence of *Escherichia coli* serogroup O 157 and the *Salmonella typhimurium* mutant DT 104 to the surface of *Saccharomyces boulardii*, Mycoses 42 (4) (1999) 261–264.
[55] A. Fasano, B. Baudry, D.W. Pumplin, S.S. Wasserman, B.D. Tall, J.M. Ketley, J.B. Kaper, Vibrio cholerae produces a second enterotoxin, which affects intestinal tight junctions 88 (12) (1991) 5242–5246.
[56] Q. Mu, J. Kirby, C.M. Reilly, X.M. Luo, Leaky gut as a danger signal for autoimmune diseases 8 (2017) 598.
[57] W. Wang, Z. Li, Q. Han, Y. Guo, B. Zhang, R. D'inca, Dietary live yeast and mannan-oligosaccharide supplementation attenuate intestinal inflammation and barrier dysfunction induced by *Escherichia coli* in broilers, Br. J. Nutr. 116 (11) (2016) 1878–1888.
[58] A. Agazzi, V. Perricone, F. Omodei Zorini, S. Sandrini, E. Mariani, X.-R. Jiang, A. Ferrari, M. Crestani, T.X. Nguyen, V. Bontempo, C. Domeneghini, G. Savoini, Dietary mannan oligosaccharides modulate gut inflammatory response and improve duodenal villi height in post-weaning piglets, Improving Feed Efficiency 10 (8) (2020) 1283.
[59] M.C. Jacobs, B.W. Haak, F. Hugenholtz, W.J. Wiersinga, Gut microbiota and host defense in critical illness, Curr. Opin. Crit. Care 23 (4) (2017).
[60] P.D. Cani, Human gut microbiome: hopes, threats and promises 67 (9) (2018) 1716–1725.
[61] A.N. Sharma, S. Kumar, A.K. Tyagi, Effects of mannan-oligosaccharides and *Lactobacillus acidophilus* supplementation on growth performance, nutrient utilization and faecal characteristics in Murrah buffalo calves, J. Anim. Physiol. Anim. Nutr. 102 (3) (2018) 679–689.
[62] I. Ofek, E.H. Beachey, Mannose binding and epithelial cell adherence of *Escherichia coli*, Infect. Immun. 22 (1) (1978) 247–254.
[63] B. Baurhoo, L. Phillip, C.A. Ruiz-Feria, Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens, Poultry Sci. 86 (6) (2007) 1070–1078.
[64] N. Browne, A. Traynor, K.A. Horgan, Mannan rich fraction from yeast modulates inflammatory responses in intestinal cells (HT-29) exposed to *Escherichia coli*, Journal of Applied Animal Nutrition 7 (2019) e5.
[65] H. Smith, S. Grant, J. Parker, R. Murphy, Yeast cell wall mannan rich fraction modulates bacterial cellular respiration potentiating antibiotic efficacy, Sci. Rep. 10 (1) (2020) 21880.
[66] G. Kogan, A. Kocher, Role of yeast cell wall polysaccharides in pig nutrition and health protection, Livest. Sci. 109 (1) (2007) 161–165.
[67] D. Hafner, Z. Bodnár, K. Horvatovich, G. Berta, M. Kovács, Preliminary Investigations into the Effect of Feeding Mannan Oligosaccharide (MOS) on the Genotoxic Effect of T-2 Toxin in Rabbits Measured by Comet Assay, 2012.
[68] S. Torrecillas, D. Montero, M.J.F. Izquierdo, s. immunology, Improved health and growth of fish fed mannan oligosaccharides: potential mode of action 36 (2) (2014) 525–544.
[69] D.W. Abbott, E.C. Martens, H.J. Gilbert, F. Cuskin, E.C.. Lowe, Coevolution of yeast mannan digestion: convergence of the civilized human diet, distal gut microbiome, and host immunity 6 (5) (2015) 334–339.
[70] A. Ganner, G.J. Schatzmayr, Biotechnology, Capability of yeast derivatives to adhere enteropathogenic bacteria and to modulate cells of the innate immune system 95 (2) (2012) 289–297.
[71] X. Jin, M. Zhang, G.-f. Cao, Y.F. Yang, *Saccharomyces cerevisiae* mannan induces sheep beta-defensin-1 expression via Dectin-2-Syk-p38 pathways in ovine ruminal epithelial cells 50 (1) (2019) 1–16.
[72] G. Gómez-Verduzco, A. Cortes-Cuevas, C. López-Coello, E. Ávila-González, G.M. Nava, Dietary supplementation of mannan-oligosaccharide enhances neonatal immune responses in chickens during natural exposure to, Eimeria spp 51 (1) (2009) 1–7.
[73] N.M. Abu-Elala, N.A. Younis, H.O. AbuBakr, N.M. Ragaa, L.L. Borges, M.A. Bonato, Efficacy of dietary yeast cell wall supplementation on the nutrition and immune response of Nile tilapia 44 (4) (2018) 333–341.

[74] H.-H. Jiang, Y.-J. Zhang, Y.-Z. Sun, R.-Q. Qi, H.-D. Chen, X.-H.J. Gao, Cell wall mannoprotein of *Candida albicans* polarizes macrophages and affects proliferation and apoptosis through activation of the Akt signal pathway 72 (2019) 308–321.
[75] T.A. Korolenko, N.P. Bgatova, V. Vetvicka, Glucan and mannan—two peas in a pod, J International journal of molecular sciences 20 (13) (2019) 3189.
[76] S.A. Ferreira, P. Pereira, P. Sampaio, P.J. Coutinho, F.M. Gama, Supramolecular assembled nanogel made of mannan 361 (1) (2011) 97–108.
[77] A.A. Adwas, A. Elsayed, A. Azab, F.A. Quwaydir, Oxidative stress and antioxidant mechanisms in human body 6 (1) (2019) 43–47.
[78] M. Schieber, N.S. Chandel, ROS function in redox signaling and oxidative stress 24 (10) (2014) R453–R462.
[79] A.R. Collins, Antioxidant intervention as a route to cancer prevention 41 (13) (2005) 1923–1930.
[80] B.N.P. Sah, T. Vasiljevic, S. McKechnie, O.N. Donkor, Effect of probiotics on antioxidant and antimutagenic activities of crude peptide extract from, yogurt 156 (2014) 264–270.
[81] H.E. Seifried, D.E. Anderson, E.I. Fisher, J.A. Milner, A review of the interaction among dietary antioxidants and reactive oxygen species 18 (9) (2007) 567–579.
[82] Y. Zhao, J. Wang, Q. Fu, H. Zhang, J. Liang, W. Xue, G. Zhao, H. Oda, Characterization and antioxidant activity of mannans from *Saccharomyces cerevisiae* with, Different Molecular Weight 27 (14) (2022) 4439.
[83] L.v. Križková, Z. Ďuračková, J. Šandula, V. Sasinková, J. Krajčovič, Antioxidative and antimutagenic activity of yeast cell wall mannans in vitro 497 (1–2) (2001) 213–222.
[84] R. Xiao, R. Power, D. Mallonee, K. Routt, L. Spangler, A. Pescatore, A. Cantor, T. Ao, J. Pierce, K.A. Dawson, Effects of yeast cell wall-derived mannan-oligosaccharides on jejunal gene expression in young broiler chickens 91 (7) (2012) 1660–1669.
[85] M. Faustino, J. Durão, C.F. Pereira, M.E. Pintado, A.P. Carvalho, Mannans and mannan oligosaccharides (MOS) from *Saccharomyces cerevisiae*, –A sustainable source of functional ingredients 272 (2021) 118467.
[86] Y. Yoshida, E. Naito, H. Mizukoshi, Y. Watanabe, K. Kimura, W. Yokoi, T. Sato, T. Okumura, M. Ito, H. Sawada, Side-chain structure of cell surface polysaccharide, mannan, affects hypocholesterolemic activity of yeast 57 (17) (2009) 8003–8009.
[87] T.A. Korolenko, T. Johnston, E. Machova, N. Bgatova, A. Lykov, N. Goncharova, Z. Nescakova, A. Shintyapina, I. Maiborodin, O.L. Karmatskikh, Hypolipidemic effect of mannans from, *C. albicans* serotypes a and B in acute hyperlipidemia in mice 107 (2018) 2385–2394.
[88] T.A. Korolenko, N.P. Bgatova, M.V. Ovsyukova, A. Shintyapina, V.J.M. Vetvicka, Hypolipidemic effects of β-glucans, mannans, and fucoidans: mechanism of action and their prospects for clinical application 25 (8) (2020) 1819.
[89] K. Hashimoto, Y. Okawa, K. Suzuki, Y. Okura, S. Suzuki, M. Suzuki, Antitumor activity of acidic mannan fraction from Bakers' yeast 6 (9) (1983) 668–676.
[90] É. Galinari, D.A. Sabry, G.L. Sassaki, G.R. Macedo, F.M.L. Passos, H.C. Mantovani, H.A.O. Rocha, Chemical structure, antiproliferative and antioxidant activities of a cell wall α-d-mannan from yeast *Kluyveromyces marxianus* 157 (2017) 1298–1305.
[91] D.R. Cameron, D.G. Cooper, R.J. Neufeld, The mannoprotein of *Saccharomyces cerevisiae* is an effective bioemulsifier 54 (6) (1988) 1420–1425.
[92] M.G. Sevag, D.B. Lackman, J. Smolens, The isolation of the components of *streptococcal* nucleoproteins in serologically active form 124 (2) (1938) 425–436.
[93] Y. Liu, G. Huang, M. Lv, Extraction, characterization and antioxidant activities of mannan from yeast cell, wall 118 (2018) 952–956.
[94] G.L. Huang, Zeitschrift für Naturforschung C, Extraction of two active polysaccharides from the yeast cell wall 63 (11–12) (2008) 919–921.
[95] J.M. Francois, A simple method for quantitative determination of polysaccharides in fungal cell walls 1 (6) (2006) 2995–3000.
[96] M. Schiavone, A. Vax, C. Formosa, H. Martin-Yken, E. Dague, J.M. François, A, combined chemical and enzymatic method to determine quantitatively the polysaccharide components in the cell wall of yeasts 14 (6) (2014) 933–947.
[97] F. Kath, W.M. Kulicke, Mild enzymatic isolation of mannan and glucan from yeast *Saccharomyces cerevisiae*, Saccharomyces cerevisiae 268 (1) (1999) 59–68.
[98] J. Li, S. Karboune, A comparative study for the isolation and characterization of mannoproteins from *Saccharomyces cerevisiae* 119 (2018) 654–661.
[99] M. Werner-Washburne, E. Braun, G.C. Johnston, R.A. Singer, Stationary phase in the yeast *Saccharomyces cerevisiae* 57 (2) (1993) 383–401.
[100] E. Valentin, E. Herrero, H. Rico, F. Miragall, R. Sentandreu, Cell wall mannoproteins during the population growth phases in *Saccharomyces cerevisiae* 148 (1987) 88–94.
[101] T. Hamada, F. Noda, K.J.A. Hayashi, Structure of cell wall and extracellular mannans from *Saccharomyces rouxii* and their relationship to a high concentration of NaCl in the growth medium 48 (4) (1984) 708–712.
[102] G. Giovani, V. Canuti, Rosi, Effect of yeast strain and fermentation conditions on the release of cell wall polysaccharides 137 (2–3) (2010) 303–307.
[103] L. Yan, K. Xia, Y. Yu, A. Miliakos, S. Chaturvedi, F. Zhang, S. Chen, V. Chaturvedi, R.J. Linhardt, Unique cell surface mannan of yeast pathogen *Candida auris* with selective binding to IgG 6 (5) (2020) 1018–1031.
[104] B. Aguilar-Uscanga, J.M. Francois, A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation 37 (3) (2003) 268–274.
[105] K. Dichtl, S. Samantaray, J.J. Wagener, Cell wall integrity signalling in human pathogenic fungi 18 (9) (2016) 1228–1238.
[106] E. Machová, P. Bystrický, A. Malovíková, S.J. Bystrický, Preparation and characterization of carboxymethyl derivatives of yeast mannans in aqueous solutions 110 (2014) 219–223.
[107] A. Saber, B. Alipour, Z. Faghfoori, A.J. Yari Khosroushahi, Cellular and molecular effects of yeast probiotics on cancer 43 (1) (2017) 96–115.
[108] P. Pais, V. Almeida, M. Yılmaz, M.C.J.J.o.F. Teixeira, *Saccharomyces boulardii*: what makes it tick as successful probiotic? 6 (2) (2020) 78.
[109] F. Ansari, S. Alian Samakkhah, A. Bahadori, S.M. Jafari, M. Ziaee, M.T. Khodayari, H.J. Pourjafar, Nutrition, Health-promoting properties of *Saccharomyces cerevisiae* var. *boulardii* as a probiotic, characteristics, isolation, and applications in dairy products 63 (4) (2023) 457–485.
[110] J.-P. Buts, Twenty-five years of research on *Saccharomyces boulardii* trophic effects: updates and perspectives 54 (1) (2009) 15–18.
[111] N.A. Da Silva, S. Srikrishnan, Introduction and expression of genes for metabolic engineering applications in *Saccharomyces cerevisiae* 12 (2) (2012) 197–214.
[112] S.N. Ande, K.R. Mudholkar, R.L. Bakal, M.D. Kshirsagar, Is *Saccharomyces boulardii* an ideal probiotic in management of gastrointestinal diseases? A review 6 (2) (2022) 78.
[113] J.C.G. Cortes, M.-Á. Curto, V.S. Carvalho, P. Perez, J.C. Ribas, The fungal cell wall as a target for the development of new antifungal therapies 37 (6) (2019) 107352.
[114] W. Schweigkofler, K. Lopandic, O. Molnár, H.J. Prillinger, Evolution, Analysis of phylogenetic relationships among Ascomycota with yeast phases using ribosomal DNA sequences and cell wall sugars 2 (1) (2002) 1–17.
[115] L.A. Parolis, H. Parolis, L. Kenne, M. Meldal, K.J.C.r. Bock, The extracellular polysaccharide of *Pichia* (*Hansenula*) *holstii*, NRRL Y-2448: the phosphorylated side chains 309 (1) (1998) 77–87.
[116] S. Weston, C.J.J.o.i. Parish, Modification of lymphocyte migration by mannans and phosphomannans, Different carbohydrate structures control entry of lymphocytes into spleen and lymph nodes 146 (12) (1991) 4180–4186.
[117] V. Ferro, C. Li, B. Wang, K. Fewings, A.R. King, E. Hammond, B.R. Creese, Synthesis of [14C]-and [35S]-labelled PI-88 for pharmacokinetic and tissue distribution studies 45 (9) (2002) 747–754.
[118] L. Lichko, T. Kulakovskaya, I.J.B. Kulaev, Extracellular phosphomannan as a phosphate reserve in the yeast *Kuraishia capsulata* 78 (2013) 674–677.
[119] N.E. Ustyuzhanina, E.V. Kulakovskaya, T.V. Kulakovskaya, V.M. Menshov, A.S. Dmitrenok, A.S. Shashkov, N.E. Nifantiev, Mannan and phosphomannan from *Kuraishia capsulata* yeast 181 (2018) 624–632.
[120] É. Galinari, J. Almeida-Lima, G.R. Macedo, H.C. Mantovani, H.A. Rocha, Antioxidant, antiproliferative, and immunostimulatory effects of cell wall α-d-mannan fractions from *Kluyveromyces marxianus* 109 (2018) 837–846.
[121] S.J. Free, Chapter two - fungal cell wall organization and biosynthesis, in: T. Friedmann, J.C. Dunlap, S.F. Goodwin (Eds.), Advances in Genetics, Academic Press, 2013, pp. 33–82.
[122] J.O. Agboola, M. Schiavone, M. Øverland, B. Morales-Lange, L. Lagos, M.Ø. Arntzen, D. Lapeña, V.G. Eijsink, S.J. Horn, L.T. Mydland, Impact of down-stream processing on functional properties of yeasts and the implications on gut health of Atlantic salmon (Salmo salar) 11 (1) (2021) 1–14.
[123] I. Ližičárová, M. Matulová, E. Machová, P. Capek, Cell wall mannan of human pathogen Candida dubliniensis 68 (1) (2007) 191–195.

[124] C.H. Ha, C.W. Yun, H.D. Paik, S.W. Kim, C.W. Kang, H.J. Hwang, H.I. Chang, Preparation and analysis of yeast cell wall mannoproteins, immune enchancing materials, from cell wall mutant *Saccharomyces cerevisiae* 16 (2) (2006) 247–255.
[125] M. Quirós, D. Gonzalez-Ramos, L. Tabera, R. Gonzalez, A new methodology to obtain wine yeast strains overproducing mannoproteins 139 (1–2) (2010) 9–14.
[126] J.C. Ribas, C. Roncero, H. Rico, A.J.F.m.l. Durán, Characterization of a *Schizosaccharomyces pombe* morphological mutant altered in the galactomannan content 79 (2–3) (1991) 263–268.
[127] P.F.-H. Lai, P.-C. Hsu, B.-K. Liou, R.D. Divate, P.-M. Wang, Y.-C. Chung, Improved, Phenolic Compositions and Sensory Attributes of Red Wines by *Saccharomyces cerevisiae* Mutant CM8 Overproducing Cell-Wall Mannoproteins 8 (11) (2020) 1483.
[128] P.W. de Groot, C. Ruiz, C.R. Vázquez de Aldana, E. Dueñas, V.J. Cid, F. Del Rey, J.M. Rodríquez-Peña, P. Pérez, A. Andel, J.J.C. Caubín, f. genomics, A genomic approach for the identification and classification of genes involved in cell wall formation and its regulation in *Saccharomyces cerevisiae* 2 (3) (2001) 124–142.
[129] N.A. Gow, J.-P. Latge, C.A.J.M.s. Munro, The fungal cell wall: structure, biosynthesis, and function 5 (3) (2017), 5.3. 01.
[130] G. Lesage, H.J.M. Bussey, Cell wall assembly in *Saccharomyces cerevisiae*, reviews 70 (2) (2006) 317–343.
[131] P.N. Lipke, R.J. Ovalle, Cell wall architecture in yeast, new structure and new challenges 180 (15) (1998) 3735–3740.
[132] A.H. Rose, The Yeasts: Metabolism and Physiology of Yeast, Academic Press, 1989.
[133] J.C. Kapteyn, H. Van Den Ende, F.M. Klis, The contribution of cell wall proteins to the organization of the yeast cell wall 1426 (2) (1999) 373–383.
[134] J. Jungmann, S.J. Munro, Multi-protein complexes in the cis Golgi of *Saccharomyces cerevisiae* with α-1, 6-mannosyltransferase activity 17 (2) (1998) 423–434.
[135] J.C. Rayner, S. Munro, Identification of the MNN2 and MNN5 mannosyltransferases required for forming and extending the mannose branches of the outer chain mannans of *Saccharomyces cerevisiae*, J. Biol. Chem. 273 (41) (1998) 26836–26843.
[136] Y.D. Lobsanov, P.A. Romero, B. Sleno, B. Yu, P. Yip, A. Herscovics, P.L. Howell, Structure of Kre2p/Mnt1p: a yeast alpha1,2-mannosyltransferase involved in mannoprotein biosynthesis, J. Biol. Chem. 279 (17) (2004) 17921–17931.
[137] S. Strahl-Bolsinger, M. Gentzsch, W. Tanner, *Protein O-mannosylation* 1426 (2) (1999) 297–307.
[138] R. Conde, G. Pablo, R. Cueva, G.J.Y. Larriba, Screening for new yeast mutants affected in mannosylphosphorylation of cell wall mannoproteins 20 (14) (2003) 1189–1211.
[139] N. Dean, Y.B. Zhang, J.B. Poster, The VRG4 gene is required for GDP-mannose transport into the lumen of the Golgi in the yeast *Saccharomyces cerevisiae*, J Biol Chem 272 (50) (1997) 31908–31914.
[140] A. Striebeck, D.A. Robinson, A.W. Schüttelkopf, D.M. van Aalten, Yeast Mnn9 is both a priming glycosyltransferase and an allosteric activator of mannan biosynthesis 3 (9) (2013) 130022.
[141] F.M. Klis, C.G. de Koster, S. Brul, Cell wall-related bionumbers and bioestimates of Saccharomyces cerevisiae and *Candida albicans* 13 (1) (2014) 2–9.
[142] M. Schiavone, N. Sieczkowski, M. Castex, E. Dague, J. Marie François, Effects of the strain background and autolysis process on the composition and biophysical properties of the cell wall from two different industrial yeasts 15 (2) (2015).
[143] M. Robert, J. Waldhauer, F. Stritt, B. Yang, M. Pauly, C. Voiniciuc, Modular biosynthesis of plant hemicellulose and its impact on yeast cells, J Biotechnology for Biofuels 14 (1) (2021) 1–17.
[144] R.F. Tester, F.H. Al-Ghazzewi, Mannans and health, with a special focus on glucomannans 50 (1) (2013) 384–391.
[145] Y. Kumagai, K. Kawakami, T. Mukaihara, M. Kimura, T.J.B. Hatanaka, The structural analysis and the role of calcium binding site for thermal stability in mannanase 94 (12) (2012) 2783–2790.
[146] M. Bozkurt, Ö. Tokuşoğlu, K. Küçükyilmaz, H. Akşit, M. Çabuk, A. Uğur Çatli, K. Seyrek, M. Çinar, Effects of dietary mannan oligosaccharide and herbal essential oil blend supplementation on performance and oxidative stability of eggs and liver in laying hens 11 (2) (2012) e41.
[147] C. Schlabitz, D.N. Lehn, C.F.V. de Souza, A Review of *Saccharomyces cerevisiae* and the Applications of its Byproducts in Dairy Cattle Feed: Trends in the Use of, Residual Brewer's Yeast 332 (2022) 130059.
[148] N. Levy-Pereira, G.S. Yasui, M.V. Cardozo, J. Dias Neto, T.H.V. Farias, R. Sakabe, S.B.d. Pádua, F. Pilarski, Immunostimulation and Increase of Intestinal Lactic Acid Bacteria with Dietary Mannan-Oligosaccharide in Nile tilapia Juveniles, 47, 2018.
[149] K.K. Schwarz, W.M. Furuya, M.R.M. Natali, M.C. Gaudezi, P.A.G.D. Lima, Mannanoligosaccharides in diets for tilapia larvae 40 (2011) 2634–2640.
[150] M.A. Genc, E. Yilmaz, E. Genc, M. Aktas, Effects of Dietary Mannan Oligosaccharides (MOS) on Growth, Body Composition, and Intestine and Liver Histology of the Hybrid tilapia (Oreochromis niloticus X 0. aureus), 59, 2007.
[151] S. Singh, G. Singh, S.K. Arya, Mannans: An overview of properties and application in food products 119 (2018) 79–95.
[152] G.S. Kaira, D. Panwar, M. Kapoor, Recombinant endo-mannanase (ManB-1601) production using agro-industrial residues: development of economical medium and application in oil extraction from, copra 209 (2016) 220–227.
[153] A. Caridi, Enological functions of parietal yeast mannoproteins, Antonie Leeuwenhoek 89 (2006) 417–422.
[154] K. Bauerova, D. Mihalova, K. Drabikova, V. Jancinova, J. Kucharska, E. Paulovicova, R. Nosá, S. Poništ, Effects of glucomannan isolated from Candida utilis on adjuvant arthritis in Lewis rats 10 (1) (2012).
[155] J.H. Hamman, Composition and applications of Aloe vera leaf gel 13 (8) (2008) 1599–1616.
[156] A.A. Abd El Tawab, F.A.-A. El-Hofy, A.M. Ammar, O.A.E.-R. Saleh, H.A. Tolba, Effect of prebiotic on the immune status of Oreochromis niloticus 30 (1) (2016) 20–28.
[157] F. Pilarski, C.A.F. de Oliveira, F.P.B.D. de Souza, F.S. Zanuzzo, Different β-glucans Improve the Growth Performance and Bacterial Resistance in Nile tilapia, 70, 2017, pp. 25–29.
[158] B. Han, K. Baruah, E. Cox, D. Vanrompay, P. Bossier, Structure-functional Activity Relationship of β-glucans from the Perspective of Immunomodulation: a Mini-Review, 11, 2020 521871.
[159] I. Avramia, S. Amariei, Spent Brewer's yeast as a source of insoluble β-glucans 22 (2) (2021) 825.
[160] S. Thammakiti, M. Suphantharika, T. Phaesuwan, C.J., Preparation of spent brewer's yeast β-glucans for potential applications in the food industry 39 (1) (2004) 21–29.
[161] M. Shalaby, M. Abo-Rya, A.-Z.M. Motawei, Effect of baking process on β-glucan content in whole barley balady bread 5 (7) (2014) 481–490.
[162] Z.E. Martins, M. Erben, A.E. Gallardo, R. Silva, I. Barbosa, O. Pinho FerreiraI.M.,, Effect of spent yeast fortification on physical parameters, volatiles and sensorial characteristics of home-made bread 50 (8) (2015) 1855–1863.
[163] Z. Martins, O. Pinho, I.J.J.o.f.s. Ferreira, Impact of New Ingredients Obtained from Brewer's Spent Yeast on Bread, Characteristics 55 (2018) 1966–1971.
[164] S. Suwannarong, R. Wongsagonsup, M. Suphantharika, Effect of Spent Brewer's Yeast β-D-glucan on Properties of Wheat Flour Dough and Bread during Chilled, Storage 156 (2020) 381–393.
[165] U. Bacha, M. Nasir, S. Iqbal, A.A. Anjum, Influence of Yeast β-Glucan on Cookies Sensory Characteristics and Bioactivities, 2018.
[166] W. Mejri, S. Bornaz, A. Sahli, Formulation of Non-fat Yogurt with β-glucan from Spent Brewer's Yeast, vol. 8, 2014, pp. 163–173.
[167] J.S. Dos Santos, V.R. de França, R.L. Venâncio, P.H. Hasegawa, A.G. de Oliveira, G.A.N. Costa, β-Glucan from *Saccharomyces cerevisiae* in skim yogurt production 35 (2019) 620–628.
[168] G. Marinescu, A. Stoicescu, L.J. Patrascu, The preparation of mayonnaise containing spent brewer's yeast, β-glucan as a fat replacer 16 (2) (2011) 6017–6025.
[169] R. De Smet, T. Demoor, S. Verschuere, M. Dullaers, G.R. Ostroff, G. Leclercq, L. Allais, C. Pilette, M. Dierendonck, B.G. De Geest, β-Glucan microparticles are good candidates for mucosal antigen delivery in oral vaccination 172 (3) (2013) 671–678.
[170] M.M. Abo Elsoud, E.M. El Kady, Current trends in fungal biosynthesis of chitin and chitosan 43 (1) (2019) 1–12.
[171] M.H. Ahmad, A. El-Mousallamy, N. Zein, A.S. Abd El-Naby, S.Z. Mohamed, Evaluation of prebiotic as natural additives on growth performance and blood biochemistry for Nile tilapia, Oreochromis niloticus) 5 (2) (2015) 526–533.

[172] K.M. Selim, R.M. Reda, Beta-glucans and mannan oligosaccharides enhance growth and immunity in Nile tilapia 77 (1) (2015) 22–30.
[173] C. Snyman, J. Mekoue Nguela, N. Sieczkowski, M. Marangon, B. Divol, Optimised extraction and preliminary characterisation of mannoproteins from non-*saccharomyces* wine yeasts 10 (5) (2021) 924.
[174] K. Bahmed, F. Quilès, R. Bonaly, J.J.B. Coulon, Fluorescence and infrared spectrometric study of cell walls from *Candida*, *Kluyveromyces*, *Rhodotorula* and *Schizosaccharomyces* yeasts in relation with their chemical composition 4 (6) (2003) 1763–1772.