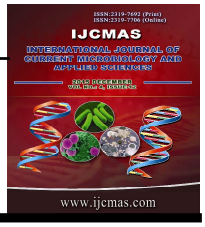
Int.J.Curr.Microbiol.App.Sci (2015) 4(12): 705-711

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 12 (2015) pp. 705-711**
http://www.ijcmas.com



Original Research Article

Partial Purification and Characterization of Mannan Oligosaccharides from Cell Wall of *Saccharomyces cerevisiae*

Vishva Maru, Supriya Hewale, Harshita Mantri and Vaijayanti Ranade*

Department of Microbiology, G. N. Khalsa College of Arts, Science and Commerce, Nathalal Parekh Marg, Matunga, Mumbai, Maharashtra, India
**Corresponding author*

Keywords

Mannan oligosaccharide, Glucan, Yeast cell wall

A B S T R A C T

Yeast cell wall consists of ß(1→3)–D–glucan, ß(1→6)–D–glucan, chitin, mannan and proteins. Mannan oligosaccharide (MOS) is a glucomannoprotein complex which can effectively bind and adsorb fimbriae of various pathogenic bacteria and thus block their colonization in the gastro intestinal tract, thereby reducing infection. In this research paper, the growth characteristics of yeast were studied on different growth media and alkali soluble mannan was extracted from yeast cell wall. The MOS carbohydrate content was found to be the highest when the culture was grown on YM media. Characterization of mannan was done using TLC and FTIR. IR spectra analysis confirmed the presence of *ν*(CH) and *ν*(CO) stretching vibrations and presence of *ν*(OH) band.

Introduction

Mannan oligosaccharide obtained from cell wall of yeast, *Saccharomyces cerevisiae* is found to inhibit colonisation of enteric pathogens such as *Salmonella, E.coli, Campylobacter*, etc. (11, 17, 22). The cell wall is mainly composed of ß(1→3)–D–glucan and ß(1→6)–D–glucan, mannoproteins and chitin. β–glucans are the main components accounting for 50–60% (by weight) of the cell wall in *S. cerevisiae* and other yeasts, while Chitin, a linear polymer of 1,4–β–linked *N*-acetyl glucosamine units is a relatively minor (1–10%), but important constituent. Rigidity and morphology of the fungal cell wall is attributed to β-glucans and chitin polymers (12). The surface properties of the cell wall are determined by mannoproteins, which represent 30–40% (by weight) of the total cell wall (15).

Mannan mainly comprises of D–mannose and in some cases additional compounds such as D–glucose/D–galactose/D–xylose and phosphate is covalently linked to protein or peptide moiety. It consists of α(1→6) linked main chain substituted by side chains of various length containing α(1→2) and α(1→3) linkages (7). Mannoprotein is linked to ß(1→6) glucan through a remnant of Glycosylphosphatidylinositol (GPI) anchor containing 5α–linked mannosyl residues. The linkage between mannoproteins and ß (1→6) glucan play a

pivotal role in organizing the yeast cell wall (6). The alkali soluble glucan is a minor component [15-20% (w/w)] of total glucan. This glucan fraction consists of ß(1→3) linked backbone containing ß(1→6) linkages which is responsible for the structural integrity of the cell wall (6).

Cell wall of yeasts and plants are the main sources of MOS. Mannans obtained from plants generally include galactomannans from guar and locust beans, glucomannan from konjac tubers, and galactoglucomannan from spruce wood as outlined in Table 1 (2, 3, 5, 8, 21).

Table.1 Sources of Mannan Oligosaccharide from Plant and Fungi

Source	Species	Type	Reference
Plant	*Ceratonia siliqua* (carob or locust bean)	Galacto mannan	Gaisford *et al.*,1986
	Caesalpinia pulcherrima		Andrade *et al.*, 1999
	Amorphophallus konjac	Gluco mannan	Chua *et al.*, 2012
	Carum carvi	Linear mannan	Hopf *et al.*, 1997
	Aloe barbadensis		Simeos *et al.*,
Fungi	*Aspergillus fumigatus*	Galactomannan	Puchart *et al.*, 2004
	Penicillium oxalicum		Kurukake *et al.*, 2006
	Saccharomyces cerevisiae	Glucomannan	Setati *et al.*, 2001
	Candida albicans	Galactomannan	Reyna *et al.*, 1999

In recent years, research interest for mannan oligosaccharides and ß–Glucan has increased substantially. *ß*–Glucan is found to stimulate macrophages of the immune system and help overcome bacterial infections. Mannan oligosaccharide provides a mannose rich source for attachment of pathogenic gut bacteria and hence prevents their colonisation in the gut (22). MOS are also found to be prebiotic and can serve as a nutrient source for growth of beneficial bacteria like *Lactobacillus and Bifidobacteria* species in the colon. Due to these benefits, MOS is also widely used in animal feed to improve gastrointestinal health and growth performance in terms of body weight gain and feed conversion in chickens, domestic animals, etc (11).

Inhibition of the adherence of *S. typhimurium* by mannose has been reported when it is added to the diet of young chicks (19). However, the concentration of mannose required to reduce colonization of pathogenic bacteria is relatively high and the cost of using pure mannose in commercial production is unsuitable. Hence, mannose–based carbohydrates serve as an alternative, since they occur naturally (22). The current research aims to extract and partially purify mannan oligosaccharide from the cell walls of *S. cerevisiae*.

Materials and Methods

Strain and Growth Conditions

S. cerevisiae strain used in this study was isolated from a food source and routinely grown on Saboraud's agar medium at ambient temperature for 48 hours. In case of liquid medium, cells were grown at shaker condition.

Growth Rate Studies

Growth curve studies of the isolated yeast culture were carried out using six different

media. Routinely used media such as Sabouraud's broth, GPB, GYEA, YPD, YM and YP_{gal} were selected for cultivation of yeast. The culture inoculated media were incubated at shaker condition for 36 hours and OD_{540nm} was checked at an interval of 2 hours up to 36 hours.

Partial Purification of Crude Mannan Oligosaccharide

Dry Cell Mass

Wet Cell Mass (WCM) was harvested by centrifugation at 6000 rpm for 15mins. Supernatant was decanted and cell pellet was washed with St. PBS. Weight of the dry cell mass was estimated in grams.

Extraction of Crude Mannan Oligosaccharides

5g yeast cell wall was treated with 50mL of 0.25N NaOH at 100°C for 2 hours, cooled and neutralized with 1N HCl to pH 7. Post centrifugation, the pellet was treated with 4 volumes of absolute ethanol. The cells were centrifuged again, pellet obtained was further washed with absolute ethanol and allowed to dry (10).

Deproteinization by Sevage Method

The dry pellet was homogenously mixed with chloroform and isoamyl alcohol (5:1(v/v)). The mixture was vigorously shaken for 10 mins. The chloroform/isoamyl layer was carefully drawn off from the residual layer. The procedure was repeated thrice. Mannan oligosaccharides obtained was washed with absolute ethanol and air dried

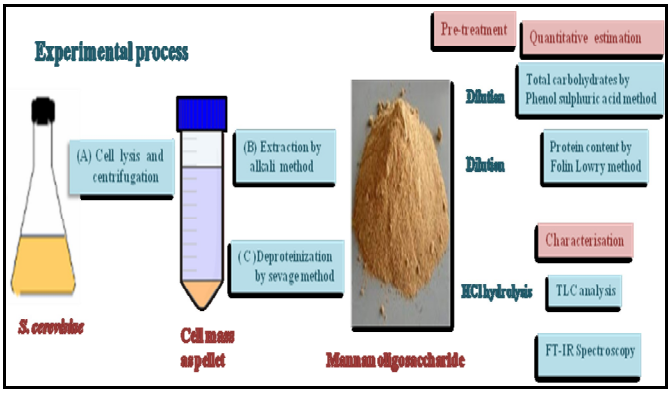
Quantitative Estimation

Total carbohydrate content of the oligosaccharide was determined by phenol-sulphuric acid method using glucose as standard (4). The protein concentration was estimated by Folin Lowry method using Bovine Serum Albumin (BSA) as standard.

Characterisation of Mannan Oligosaccharide

Mannan was hydrolysed under mildly acidic condition with 1N HCl for up to 6 hours at 100ºC. The outcome of hydrolysis was analysed by thin layer chromatography using silica gel coated aluminium sheets (Merck) with butanol: acetic acid: water (2:1:1) as the solvent system. Hydrolysis products were detected using aniline phthalate developer by heating the sheets for 10mins at 100ºC (20). IR spectra were recorded with an FT-IR apparatus IR Affinity 1S, of Shimadzu and wave numbers were reported in cm^{-1}. The sample in dry form was directly placed on the sample holder for analysis.

Figure.1 Overview of Experimental Process

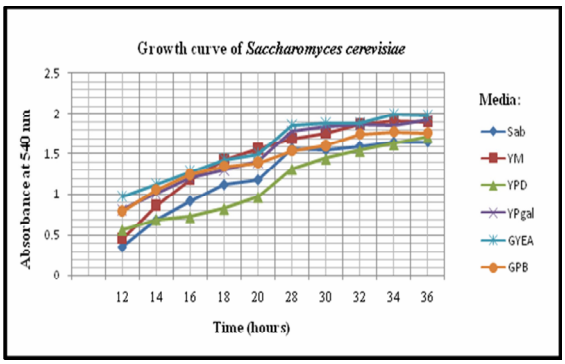


Results and Discussion

Yeast being a GRAS product is safe to use and easy to cultivate. Growth curve was performed in order to study the effect of media constituents on the growth and generation time of the culture. As per the shake flask data, GYEA and YPgal were found to show maximum growth for yeast as compared to other media. Carbon and

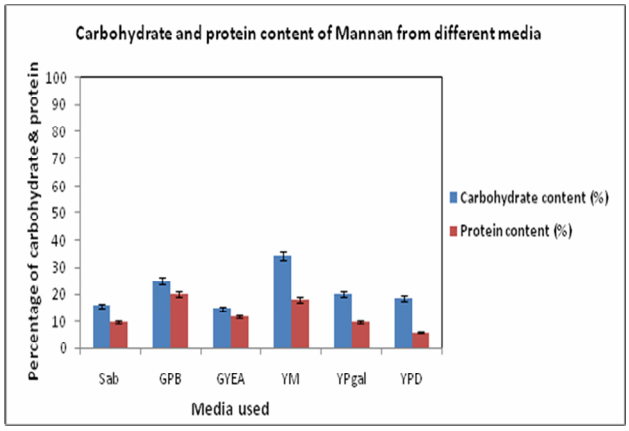
nitrogen source when provided in adequate quantities along with sufficient aeration allows aerobic respiration in yeasts which results in increased biomass.

Figure.2 Growth Curve of *Saccharomyces Cerevisiae* using Different Media



MOS obtained from yeast cultivated on YM medium showed highest carbohydrate content of 30% followed by GPB of 25%. However, the protein content for MOS from YM medium was found to be 18% and that from GPB was 20% as shown in figure 3. Our data is in accordance with the data obtained by White et al (23). MOS content in commercially available sources such as that from Agrimos, Advanta, etc. is found to be in the range of 26-33%. Alkali extraction was selected as the method of choice, since it extracts larger amounts of mannan as mentioned by Nelson et al 1991(16).

Figure.3 Carbohydrate and Protein Content of Mannan from Different Media



On TLC plate, the hydrolysed product migrated with R_f value similar to that of glucose and mannose. Mannans obtained from yeasts are generally glucomannan (13) as opposed to galactomannans found in some plants.

The IR spectrum of mannan oligosaccharides shown in Figure 3 shows absorption bands arising from the *v*(CH) and the *v*(CO) stretching vibrations and high intensity of the *v*(OH) band. The presence of a carbonyl group and amide bond clearly suggests that Mannan oligosaccharides are tightly associated with proteins and together they form mannoprotein complex.

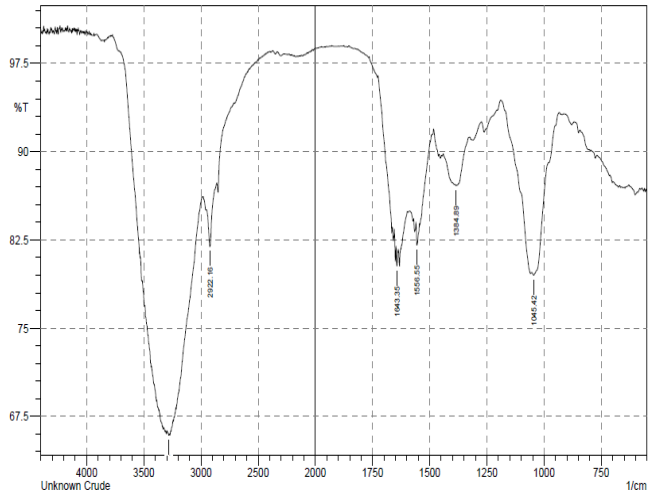
Probable functional groups represented by each band are explained in table 2. Commercially available MOS of Praj Industries Ltd, Pune was used as standard. FTIR data of extracted sample was found to be quite similar with that of the standard.

Alkali extraction is thus a quite simple, inexpensive and less time consuming method of extraction. Mannan extracted from yeast has various applications such as use as a prebiotic, fermentative additive, immunomodulator etc.

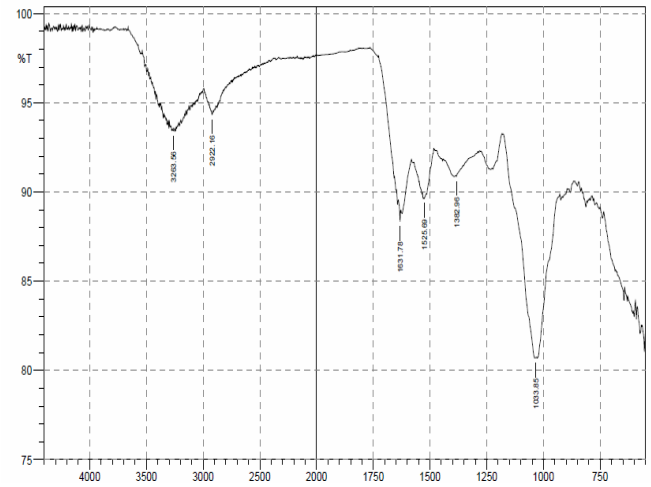
Table.2 Probable Functional Groups of Peak Obtained in FTIR

Peak Wave number (cm-1)	Probable Functional groups
3234.62	O-H Stretching
2897.08	C-H Stretching (Alkane)
1643.35	C=O Stretching (attached to Amide)
1622.13	N-H Stretching (Amide)
1529.55	N-H Stretching (Amide)
1039.63	C-O Stretching (Alcohol)

Figure.4 FTIR Spectra of (a) Extracted Mannan Oligosaccharide, (b) Standard MOS



(a)



(b)

Acknowledgement

The authors are grateful to Vivekananda Education Society's College of Pharmacy, Chembur for FTIR facility, Dr. Ajit Datar, GNIRD faculty and Praj Industries Pvt Ltd for providing MOS sample.

Reference

1. Aguilar-Uscanga, B and Francois, J.M. (2003) A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation. Letters in Applied Microbiology, 37: 268–274.
2. Andrade , C.T., Azero, E., Luciano, L., Goncalves, M.(1999) Solution properties of the galactomannans extracted from the seeds of *Caesalpinia pulcherrima* and *Cassia jaanica*: comparison with locust bean gum. International Journal of Biological Macromolecules, 26:181–185
3. Chuaa, M., Chana, K., Hockinga, T., Williams, P., Perrya, C. and Baldwina, M., (2012) Methodologies for the extraction and analysis of konjac glucomannan from corms of

Amorphophallus konjac. Carbohydrate Polymers 87, 2202– 2210.
4. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith (1956) Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.
5. Gaisford, S., Harding, S., Mitchellt , J. and Bradley, T. (1986) A Comparison Between the Hot and Cold Water Soluble Fractions of Two Locust Bean Gum Samples. *Carbohydrate Polymers* 6: 423-442.
6. Ha, C., Lim, K., Kim, Y., Lim, S., Kim, C. and Chang, H. (2002) Analysis of alkali-soluble glucan produced by *Saccharomyces cerevisiae* wild-type and mutants. Appl. Microbiol Biotechnol, 58: 370–377.
7. Hamada, T., Nakajima, T., Izaki, K., Matsuda, K.(1981) Comparison of the Mannan Structure from the Cell-Wall Mutant *Candida* sp. M-7002 and Its Wild Type. Eur. J. Biochem. 119: 365-371.
8. Hopf, H. and Kandler, O. (1997) Characterization of the 'reserve cellulose' of the endosperm of Carum Carvi as a ß (l-4)-mannan. Phytochemistry 16: 715-717.
9. Huang, G. (2008) Extraction of Two Active Polysaccharides from the Yeast Cell Wall, Z. Naturforsch.
10. Huang, G., Yang, Q. and Wang, Z. (2010) Extraction and Deproteinization of Mannan Oligosaccharides, Z. Naturforsch.
11. Koc, F., Samli, H., Okur, A., Ozduven, M., Akyurek, H. and Senkoylu, N., (2010) Effects of *Saccharomyces cerevisiae* and/or Mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. Bulgarian Journal of Agricultural Science, 16: 643-650.
12. Kollar, R., et al (1997) Architecture of the Yeast Cell Wall. J. Biol. Chem 272:17762–17775.
13. Kocourek, J. and Ballou, C. (1969) Yeast Cell Wall Mannans, *J. Bacteriol*, 100(3):1175.
14. Levitz, S.(2010) Innate Recognition of Fungal Cell Walls, PLoS Pathog 6
15. Moreno, I., Tutrone, N., Sentandreu, R., Valentín, E. (2008) *Saccharomyces cerevisiae* Rds2 transcription factor involvement in cell wall composition and architecture. International Microbiology 11:57-63.
16. Nelson, R., Shibata, N., Podzorski, R. and Herron, M.,(1991) Candida Mannan: Chemistry, Suppression of Cell-Mediated Immunity, and Possible Mechanisms of Action ,Clinical Microbiology reviews, Vol. 4, No. 1, p1-19.
17. Newman, K., 1994. Biotechnology in the Feed Industry. Proc. Alltech's 10th Annu. Symp. T. P. Lyons and K. A. Jacques, (eds.) Nottingham Univ. Press, Nottingham, UK. Mannan-Oligosaccharides: Natural Polymers with Significant Impact on the Gastrointestinal Microflora and the Immune System. Pp. 167-174.
18. Noha, Mohamed, M.A., Sedki, A.A., Faten, Ibrahim, A.A. and EL-Kaiaty, A.M., (2013)The Effects of Dietary Mannanoligosaccharides on Cecal Parameters and the Concentrations of Enteric Bacteria in the Ceca of Salmonella-Challenged Broiler Chicks. Poultry Science 79:205–211.
19. Oyofo, B. A., J. R. DeLoach, D. E. Corrier, J. O. Norman, R. L. Ziprin, and H. H. Mollenhauer, 1989. Effect of carbohydrates on *Salmonella typhimurium* colonization in broiler chickens. Avian Dis. 33:531–534.
20. Ranta, K., Nieminen, K., Saariaho, T., Kortekangas-Savolainen, O., Kumpula, E., Kosonen, J., Pasanen, A., Savolainen,

J. (2013) Evaluation of Fungal Extracts to Determine Immunomodulatory Properties. J Investig Allergol Clin Immunol 23:226-233.

21. Simoesa, J., Nunes, F., Dominguesa,P., Coimbraa,M., Domingues, M.R.(2012) Mass spectrometry characterization of an Aloe vera mannan presenting immunostimulatory activity. Carbohydrate Polymers 90: 229– 236.
22. Spring, P., Wenk, C., Dawson, K. and Newman, K. (2000) Effect of sodium gluconate, mannan oligosaccharide and potassium diformate on performance of growing New Zealand white rabbits. Egypt. Poult. Sci. Vol 33: 73-85.
23. White, L., Newman, M., Cromwell, G. and Lindemann, M. (2002) Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. *J anim sci* 80:2619-2628.
24. Yildiz, S. and Oner ,E. Mannan as a Promising Bioactive Material for Drug Nanocarrier Systems Application of Nanotechnology in Drug Delivery.