OPTIMISATION OF THE EXTRACTION PROCESS AND PARTIAL CHARACTERIZATION OF B-GLUCAN FROM THE MARINE YEAST *DEBARYOMYCES HANSENII*.

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ABSTRACT

The cell walls of fungi are made with structural polysaccharides as well as glycoproteins. They are the main source of various glucans. *Debaryomyces hansenii* yeast cells (15% W/V) was used as the source of β- glucan extraction. 1M NaOH (sodium Hydroxide) / 1M HCl , 1 M NaOH / 1M CH3COOH , 1 M NaOH / 1M H3PO4 combinations were used for optimization of the extraction procedure. Maximum yield of β-glucan from *D. hansenii* was obtained with a combination of 1M NaoH / 1M H3PO4 extraction method. FT-IR analysis of the extracted β-glucan identified the signature peeks of bonds (1,3) and (1,6)-β-D-glucan linkages.

Keywords: *Debaryomyces hansenii*, Marine yeast, β-glucan, extraction, optimization.

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1. INTRODUCTION

The dry mass of yeast is made up of 15% - 30% of the cell wall contents. The cell wall chemical composition was well elucidated (Aguilar-Uscanga & Francois, 2003) and is principally composed of manno-proteins and L-glucans which accounts for 85-90% of the total cell wall dry mass. Minor quantity ie.,1-3% of chitin and 2-5% of lipids are also present (Nguyen et al., 1998; Thammakiti et al., 2004). Yeast cell comprises of dual layers, the first layer is nearer to the cell membrane and the second layer lies towards the surface of the cell. The chief polysaccharide present in the inner layer of yeast cell wall is 1→3 β-glucan with 1→6 branches (Fleet, 1985; Kollár et al., 1997; Lipke & Ovalle, 1998; Ovalle et al., 1998; Peat et al., 1961). β-glucan formulates continuous 3-D complex layer which is much elastic and it maintains cell shape and protects cell from osmotic pressure fluctuations. Mannoproteins, are a kind of O and N glycosylated polypeptides and are located on the outer region of the cell wall (De Nobel et al., 1990). Their function is to limit the permeability of the cell wall towards the solute (Worrasinchai et al., 2006). The cell walls of fungi are made with structural polysaccharides as well as glycoproteins. They are the main source of various glucans. Types of glucans are determined by the type of bond present in between the individual sugar units. β- Glucans with mainly three types of bonds are usually found in fungal cells and varies with species *viz*. β-(1→3)- and/or β-(1→6)-linked β-D-glucan units, and β-(1→4)- linked β-D-glucan units may be present (Zechner-Krpan et al., 2010). β-glucans are named with several trivial names based on the source of fungal species (Šandula et al., 1999). Grifolan, lentinan, pachyman, pleuran, schizophylan, scleroglucan, etc., are the few types OF β-glucans (Kenyon & Buller, 2002; Novak & Vetvicka, 2008).

As β-glucan is located in the cell wall, it is essential to break open/lyse the cell and separate the cell wall from the cytoplasm of the yeast cell (Ohno et al., 1999). β-glucan in yeast is a complex structure with branched units and is a water insoluble component. It contains 1→3 and 1→6 linkages between the residues (Hunter et al., 2002). Various chemical, physical and enzymatic methods were described by several workers and chemicals like NaOH, HCl, acetic acid, citric acid chemical solutions, physical disruption by means of sonication, homogenizers with high pressure, etc. are commonly followed (Freimund et al., 2005). Out of all methods, autolysis which is done by altering the physical parameters like bringing change in the osmolarity of the solution followed by the alkali and / or acid extraction was reported to be the reliable method of β-glucan extraction (Suphantharika et al., 2003).

Structural analysis of polysaccharides is most commonly done by FTIR spectroscopy. FTIR spectroscopic method identifies the position and anomeric pattern of glycosidic bonds in glucans. Using this tool it is possible to analyze glucans in crude and high molecular fractions isolated from various sources (Chen et al., 1998; Kümmerle et al., 1998). FTIR analyses different glucans such as branched (1→3, 1→6) as well as linear (1→3) glucans. It also recognises the fractions of other cell wall components such as proteins and can be used to establish relationships such as ptotein to glucan ratio (Galichet, 2001; Pengkumsri et al., 2017). Several workers reported the use of FTIR spectroscopy to differentiate several fungal glucans in polysaccharide fractions from various sources for different uses (Amparyup et al., 2013; Bilej et al., 2001; Lebron et al., 2003; Ooi & Liu, 2000; Raa, 2015; Roux et al., 2002).

This work deals with the optimization of the β-glucan extraction process from the marine yeast *Debaryomyces hansenii* and partial characterization of the β-glucan through FT-IR analysis.

2. MATERIALS AND METHODS

2.1 Manual extraction of β-glucan

2.1.1 Autolysis step

D. hansenii cell suspension (15% W/V) in distilled water was the source of β- glucan. In the Initiation step, the cell suspension was subjected to incubation at 50 ^{O}C and pH 5.0 (adjusted with 1M HCl) for 48 hours (shaking at 120 rpm). During termination step, the cell suspension was incubated at 80 ^{O}C for 15 min (deactivation of endolytic enzymes). It was followed by the centrifugation at 5000 rpm for 10 min, pellets were dried at 60 ^{O}C and stored at 4 ^{O}C until glucan extraction (Liu et al., 2008).

2.1.2. Extraction chemicals

Experimental design for optimisation of β- glucan extraction Method (Zechner-Krpan et al., 2010).

1M NaOH (sodium Hydroxide) / 1M HCl (Hydro chloric Acid) extraction

1M NaOH (Sodium Hydroxide) / 1M CH_3COOH (Acetic Acid) extraction

1M NaOH (Sodium Hydroxide) / 1M H_3PO4 (Phosphoric acid) extraction

2.1.3 Extraction Procedure

During 'Base treatment', 1 part of autolyzed dry yeast was mixed with 10 parts of base and

incubated at 80 ^{o}C for 2 h at constant stirring. Suspension was centrifuged at 5000 rpm for 15 min, supernatant was discarded and pellet was collected. This was followed by the 'Washing step' The pellet was suspended in 5 parts of distilled water, mixed well and centrifuged at 5000 rpm for 15 min and pellet was collected. During the step of 'Acid treatment', Pellet was dissolved in 10 parts of acid and Incubated at 80^{o}C for 2 h at constant stirring. Suspension was centrifuged at 5000 rpm for 15 min, supernatant was discarded and pellet was collected followed by the washing step. This was further followed by the 'Ethanol treatment'**,** in which, the pellet was suspended in 5 parts of ethanol and boiled at 50 ^{o}C at constant stirring until the volume of ethanol reduced to half. The Suspension was centrifuged at 5000 rpm for 15 min, supernatant was discarded and pellet was collected. This was followed by the 'Washing step'**.** The pellet was dried in hot air oven at 60 ^{O}C and stored at 4 ^{O}C until use (Suphantharika et al., 2003).

2.1.4 Glucan assay

The β-glucan obtained through extraction process was estimated by using yeast glucan assay kit (Megazyme, Ireland) as described in section 2.2.5 and contents of β-glucan was expressed in g/100g of dry weight (dry weight basis).

2.1.5 Infrared Spectroscopy

The FT-IR (Fourier-transform infrared spectroscopy) analysis of the samples was carried through FT-IR-8300 instrument (Shimadzu). The dried samples were mixed with KBr powder (glucan/KBr ratio of 2/200 mg) and transparent blend in the form a film/tablet was made by applying 10 tons of pressure using hydraulic piston. FT-IR spectrum was recorded at a resolution of 2 cm^{-1} between 4000 cm^{-1} and 400 cm^{-1}. A total of 32 scans were performed (Szymanska-Chargot & Zdunek, 2013).

3. RESULTS AND DISCUSSION

3.1 Optimisation of extraction process of β-glucan from *D.hansenii*

The suspension of 15%w/v of yeast cells in distilled water was subjected to autolysis (pH 5.0) followed by extraction using the following combination of acids and bases (Table.1)

Table.1: Extraction of β- glucan by using different extraction methods

Extraction Method	**β-glucan (g/100g)***
1M NaoH / 1M HCl	61.41 ± 1.15
1M NaoH / 1M CH_3COOH	67.32 ± 1.57
1M NaoH / 1M H_3PO_4	71.16 ± 2.12

*Dry weight basis

Different acids were evaluated for the extraction of glucan form the marine yeast, maximum amount of β-glucan (71.16 ± 2.12 g/100g) was obtained when extracted with 1M NaoH / 1M H_3PO_4. (Pengkumsri et al., 2017) also used similar method to extract maximum levels of β-glucan from a yeast strain by using the combination of a strong base (NaOH) and weak acid (CH_3COOH). Where as, in this study, maximum glucan was obtained in the combination of NaoH and H_3PO_4.

3.2 FT-IR

FT-IR spectrum elucidated that the extracted compound contains (1,3)-(1,6)-β-glucan with small concentrations of proteins and mannans as impurities (Fig.1). In 4000 – 3000 Cm^{-1} band range represents the vibration stretch of OH groups, the peaks at 3451 Cm^{-1} and 3371 Cm^{-1} obtained for commercial (1,3)-(1,6)-β-glucan from yeast and Glucan extracted from marine yeast respectively elucidates the presence of OH groups (Table. 2) . In 3000-2840 Cm^{-1} band range, the peaks at 2920 and 2923 for commercial (1,3)-(1,6)-β-glucan and for Glucan extracted from marine yeast represents the presence of CH and CH_2 stretching (CH_2OH). (Zechner-Krpan et al., 2010) while working on Characterization of β-glucans isolated from Brewer's Yeast and dried by different methods reported that free hydroxyl groups absorb in the region of 3650–3500 cm–1. The hydroxyl groups, which participate in the formation of hydrogen bond, caused shifting of the band maximum position to lower frequencies, increasing intensity, as well as broadening of the band, but causing its symmetry distortion at the same time.

In 1200-950 Cm^{-1} band range, which is the characteristic for polysaccharides, the peaks were obtained at 1142,1068,1051 Cm^{-1} for commercial (1,3)-(1,6)-β-glucan and 1080, 1040, 1168 Cm^{-1} for Glucan extracted from Marine Yeast represents the COC and CC stretching vibrations. (Hromaadkova et al., 2003) , reported that the absorption bands coming from n (C–C) and n (C–O–C) showed vibration broadening at 1160 cm^{-1}.

The band range 950-750 Cm $^{-1}$ are the complex skeletal vibrations especially represents the region for Mannans and global glucan content. 895 Cm $^{-1}$ peak for Commercial (1,3)-(1,6)-β-glucan and 891 Cm $^{-1}$ peak for extracted Glucan represents the presence of β - Glycosidic bond (Glucan). Where as the peaks at 766 Cm $^{-1}$ and 761 Cm $^{-1}$ for Commercial and extracted glucan respectively represents the presence of impurities of Mannans with α - Glycosidic bond. Similar results were reported by (Kenyon & Buller, 2002; Schmid et al., 2001). The peaks at 1634, 1543 Cm $^{-1}$ and 1651, 1546 Cm $^{-1}$ for commercial and extracted glucan respectively represents the presence of impurities of proteins (amide linkages) CN and NH groups. (Piotrowska & Masek, 2015) worked on the glucan extraction from *Saccharomyces cerevisiae* cell wall components by alkaline and water extraction methods and reported protein residues in the final product which was evidenced by the bands at 1400–1700 cm^{-1}.

Table. 2: Inference of FT IR spectrum of commercial and extracted β- glucan

Band range (Cm $^{-1}$)	Wavelength recorded		Inference
	Commercial (1,3)-(1,6)-β-glucan from yeast (Cm $^{-1}$)	Glucan extracted from Marine Yeast (Cm $^{-1}$)	
4000 - 3000	3451	3371	Vibration stretch of OH groups
3000-2840	2920	2923	CH and CH2 stretching (CH2OH)
1200-950 Cm $^{-1}$ (characteristic for polysaccharides)	1142,1068,1051	1080, 1040, 1168	COC and CC stretching vibrations
950-750 (Complex skeletal vibrations : region for Mannans and global glucan content)	895	891	β - Glycosidic bond (Glucan)
	Peaks around 1160, 1080, 1044, 890 are characteristic for (1,3)-(1,6)-β-glucan		
	766	761	α - Glycosidic bond (Mannans)
Protein contamination	1634, 1543	1651, 1546	CN and NH groups of the proteins (amide linkages).

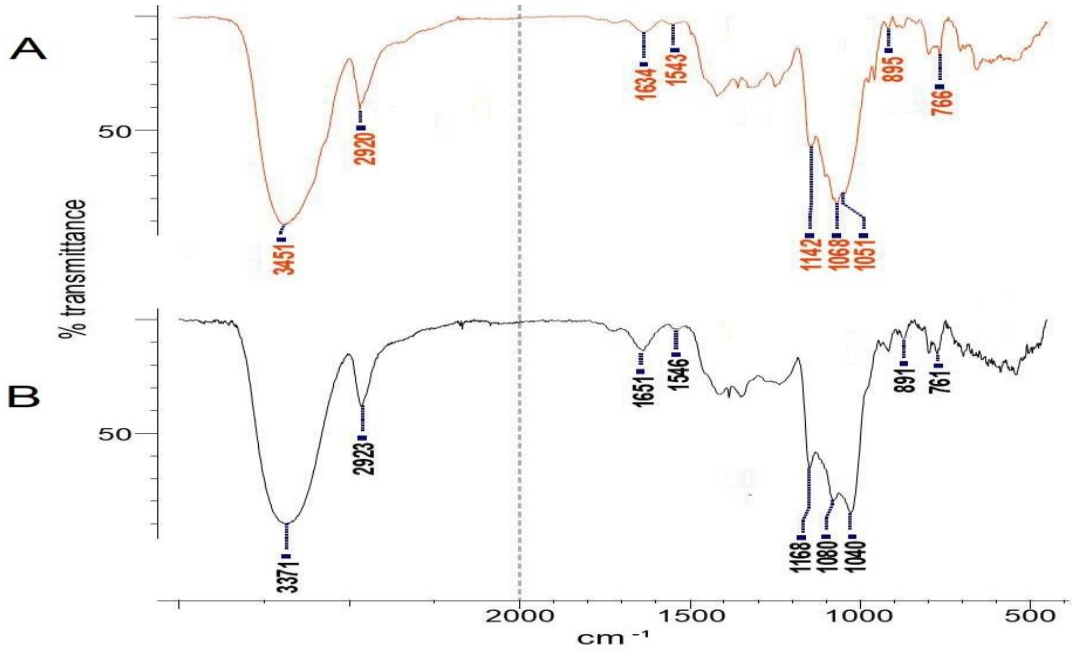

Fig.1: FT-IR Spectrum, A: commercial yeast β-Glucan, B: β- glucan extracted from marine yeast.

4. CONCLUSIONS

Through optimisation studies of extraction procedure, it was found that maximum extraction of β-glucan from *Debaryomyces hansenii* was done by using 1M NaoH / 1M H3PO4 . FT-IR analysis of the extracted β-glucan showed the signature peeks of bonds (1,3) and (1,6)-β-D-glucan linkages. Thus it can be concluded that the type of

beta-glucan present in *Debaryomyces hansenii* is of (1,3)-(1,6)-β-glucan.

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